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* * * * * * * * * *  Welcome to STN International  * * * * * * * * * *

 NEWS  1          Web Page URLs for STN Seminar Schedule - N. America
 NEWS  2          "Ask CAS" for self-help around the clock
 NEWS  3  FEB 28  PATDPAFULL - New display fields provide for legal status
                  data from INPADOC
 NEWS  4  FEB 28  BABS - Current-awareness alerts (SDIs) available
 NEWS  5  MAR 02  GBFULL: New full-text patent database on STN
 NEWS  6  MAR 03  REGISTRY/ZREGISTRY - Sequence annotations enhanced
 NEWS  7  MAR 03  MEDLINE file segment of TOXCENTER reloaded
 NEWS  8  MAR 22  KOREAPAT now updated monthly; patent information enhanced
 NEWS  9  MAR 22  Original IDE display format returns to REGISTRY/ZREGISTRY
 NEWS  10 MAR 22  PATDPASPC - New patent database available
 NEWS  11 MAR 22  REGISTRY/ZREGISTRY enhanced with experimental property tags
 NEWS  12 APR 04  EPFULL enhanced with additional patent information and new
                  fields
 NEWS  13 APR 04  EMBASE - Database reloaded and enhanced
 NEWS  14 APR 18  New CAS Information Use Policies available online
 NEWS  15 APR 25  Patent searching, including current-awareness alerts (SDIs),
                  based on application date in CA/CAplus and USPATFULL/USPAT2
                  may be affected by a change in filing date for U.S.
                  applications.
 NEWS  16 APR 28  Improved searching of U.S. Patent Classifications for
                  U.S. patent records in CA/CAplus
 NEWS  17 MAY 23  GBFULL enhanced with patent drawing images
 NEWS  18 MAY 23  REGISTRY has been enhanced with source information from
                  CHEMCATS
 NEWS  19 JUN 06  STN Patent Forums to be held in June 2005
 NEWS  20 JUN 06  The Analysis Edition of STN Express with Discover!
                  (Version 8.0 for Windows) now available
 NEWS  21 JUN 13  RUSSIAPAT: New full-text patent database on STN
 NEWS  22 JUN 13  FRFULL enhanced with patent drawing images
 NEWS  23 JUN 20  MEDICONF to be removed from STN

 NEWS EXPRESS  JUNE 13 CURRENT WINDOWS VERSION IS V8.0, CURRENT
               MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP),
               AND CURRENT DISCOVER FILE IS DATED 13 JUNE 2005

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 NEWS PHONE    Direct Dial and Telecommunication Network Access to STN
 NEWS WWW      CAS World Wide Web Site (general information)

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FILE 'HOME' ENTERED AT 18:36:36 ON 22 JUN 2005

=> file uspatful
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                       ENTRY    SESSION
FULL ESTIMATED COST                                     0.21       0.21

FILE 'USPATFULL' ENTERED AT 18:36:44 ON 22 JUN 2005
CA INDEXING COPYRIGHT (C) 2005 AMERICAN CHEMICAL SOCIETY (ACS)

FILE COVERS 1971 TO PATENT PUBLICATION DATE: 21 Jun 2005 (20050621/PD)
FILE LAST UPDATED: 21 Jun 2005 (20050621/ED)
HIGHEST GRANTED PATENT NUMBER: US6910221
HIGHEST APPLICATION PUBLICATION NUMBER: US2005132458
CA INDEXING IS CURRENT THROUGH 21 Jun 2005 (20050621/UPCA)
ISSUE CLASS FIELDS (/INCL) CURRENT THROUGH: 21 Jun 2005 (20050621/PD)
REVISED CLASS FIELDS (/NCL) LAST RELOADED: Apr 2005
USPTO MANUAL OF CLASSIFICATIONS THESAURUS ISSUE DATE: Apr 2005

>>>  USPAT2 is now available.  USPATFULL contains full text of the     <<<
>>>  original, i.e., the earliest published granted patents or         <<<
>>>  applications.  USPAT2 contains full text of the latest US         <<<
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>>> publications, starting in 2001, for the inventions covered in <<<
>>> USPATFULL. A USPATFULL record contains not only the original <<<
>>> published document but also a list of any subsequent <<<
>>> publications. The publication number, patent kind code, and <<<
>>> publication date for all the US publications for an invention <<<
>>> are displayed in the PI (Patent Information) field of USPATFULL <<<
>>> records and may be searched in standard search fields, e.g., /PN, <<<
>>> /PK, etc. <<<

>>> USPATFULL and USPAT2 can be accessed and searched together <<<
>>> through the new cluster USPATALL. Type FILE USPATALL to <<<
>>> enter this cluster. <<<
>>> <<<
>>> Use USPATALL when searching terms such as patent assignees, <<<
>>> classifications, or claims, that may potentially change from <<<
>>> the earliest to the latest publication. <<<

This file contains CAS Registry Numbers for easy and accurate
substance identification.

=> s hydroxyapatite
L1 9532 HYDROXYAPATITE

=> s l1 and hydroxyapatite/clm
1599 HYDROXYAPATITE/CLM
L2 1599 L1 AND HYDROXYAPATITE/CLM

=> s l2 and adenovir?
33013 ADENOVIR?
L3 69 L2 AND ADENOVIR?

=> s l3 and adenovir?/clm
2978 ADENOVIR?/CLM
L4 13 L3 AND ADENOVIR?/CLM

=> d l4,cbib,1-13

L4 ANSWER 1 OF 13 USPATFULL on STN
2005:104948 Producing telomerase activators and inhibitors by screening with purified telomerase.
Weinrich, Scott L., Chesterfield, MO, UNITED STATES
Atkinson, Edward M. III, Seattle, WA, UNITED STATES
Lichtsteiner, Serge P., Encinitas, CA, UNITED STATES
Vasserot, Alain P., Berkeley, CA, UNITED STATES
Pruzan, Ronald A., Palo Alto, CA, UNITED STATES
US 2005089883 A1 20050428
APPLICATION: US 2004-811033 A1 20040326 (10)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 2 OF 13 USPATFULL on STN
2004:204153 Antibodies associated with alterations in bone density.
Brunkow, Mary E., Seattle, WA, UNITED STATES
Galas, David J., Claremont, CA, UNITED STATES
Kovacevich, Brian, Renton, WA, UNITED STATES
Mulligan, John T., Seattle, WA, UNITED STATES
Paeper, Bryan W., Seattle, WA, UNITED STATES
Van Ness, Jeffrey, Claremont, CA, UNITED STATES
Winkler, David G., Seattle, WA, UNITED STATES
Darwin Discovery Ltd., Bothell, WA, UNITED STATES, 98021 (U.S. corporation)
US 2004158045 A1 20040812
APPLICATION: US 2004-788606 A1 20040227 (10)
PRIORITY: US 1998-110283P 19981127 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 3 OF 13 USPATFULL on STN
2004:76543 Compositions and methods for increasing bone mineralization.
Brunkow, Mary E., Seattle, WA, UNITED STATES
Galas, David J., Claremont, CA, UNITED STATES
Kovacevich, Brian, Renton, WA, UNITED STATES
Mulligan, John T., Seattle, WA, UNITED STATES
Paeper, Bryan, Seattle, WA, UNITED STATES
Van Ness, Jeffrey, Claremont, CA, UNITED STATES
Winkler, David G., Seattle, WA, UNITED STATES
Darwin Discovery Ltd., Slough, UNITED KINGDOM, SL1 3WE (U.S. corporation)
US 2004058321 A1 20040325
APPLICATION: US 2002-95248 A1 20020307 (10)
PRIORITY: US 1998-110283P 19981127 (60)

DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 4 OF 13 USPATFULL on STN
2003:238090 Compositions and methods for increasing bone mineralization.
Brunkow, Mary E., Seattle, WA, UNITED STATES
Galas, David J., Claremont, CA, UNITED STATES
Kovacevich, Brian, Renton, WA, UNITED STATES
Mulligan, John T., Seattle, WA, UNITED STATES
Paeper, Bryan W., Seattle, WA, UNITED STATES
Van Ness, Jeffrey, Claremont, CA, UNITED STATES
Winkler, David G., Seattle, WA, UNITED STATES
Darwin Discovery Ltd., Bothell, WA, UNITED STATES (U.S. corporation)
US 2003166247 A1 20030904
APPLICATION: US 2003-384893 A1 20030306 (10)
PRIORITY: US 1998-110283P 19981127 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 5 OF 13 USPATFULL on STN
2003:214336 Traversal of nucleic acid molecules through a fluid space and expression in repair cells.
Sosnowski, Barbara A., Coronado, CA, UNITED STATES
Pierce, Glenn, Rancho Santa Fe, CA, UNITED STATES
Selective Genetics, Inc., San Diego, CA, UNITED STATES, 92121 (U.S. corporation)
US 2003148979 A1 20030807
APPLICATION: US 2002-264284 A1 20021002 (10)
PRIORITY: US 2001-327513P 20011003 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 6 OF 13 USPATFULL on STN
2003:10286 Urease-based vaccine and treatment for helicobacter infection.
Michetti, Pierre, Lausanne, SWITZERLAND
Corthesy-Theulaz, Irene, Lausanne, SWITZERLAND
Blum, Andre, Romammotier, SWITZERLAND
Davin, Catherine, Rule de Moulins, FRANCE
Haas, Rainer, Tubingen, GERMANY, FEDERAL REPUBLIC OF
Kraehenbuhl, Jean-Pierre, Rivaz, SWITZERLAND
Saraga, Emilia, Lausanne, SWITZERLAND
US 2003007980 A1 20030109
APPLICATION: US 2001-955739 A1 20010918 (9)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 7 OF 13 USPATFULL on STN
2002:126348 Method for purifying **adenoviruses**.
Cannon-Carlson, Susan V., Wayne, NJ, UNITED STATES
Cutler, Collette, Bloomingdale, NJ, UNITED STATES
Vellekamp, Gary J., Glen Ridge, NJ, UNITED STATES
Voloch, Marcio, New York, NY, UNITED STATES
Schering Corporation (U.S. corporation)
US 2002064860 A1 20020530
APPLICATION: US 2001-991080 A1 20011116 (9)
PRIORITY: US 2000-253823P 20001129 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 8 OF 13 USPATFULL on STN
2001:212417 In situ bioreactors and methods of use thereof.
Pierce, Glenn, Rancho Santa Fe, CA, United States
Chandler, Lois Ann, Encinitas, CA, United States
US 2001044413 A1 20011122
APPLICATION: US 2000-729644 A1 20001130 (9)
PRIORITY: US 1999-168470P 19991201 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 9 OF 13 USPATFULL on STN
2001:157800 Urease-based vaccine and treatment for helicobacter infection.
Michetti, Pierre, Lausanne, Switzerland
Corthesy-Theulaz, Irene, Lausanne, Switzerland
Blum, Andre, Romammotier, Switzerland
Davin, Catherine, Nyon, Switzerland
Haas, Rainier, Tubingen, Switzerland
Kraehenbuhl, Jean-Pierre, Rivat, Switzerland
Saraga, Emilia, Lausanne, Switzerland
OraVax, Inc., Cambridge, MA, United States (U.S. corporation)

US 6290962 B1 20010918
APPLICATION: US 1994-200346 19940223 (8)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 10 OF 13 USPATFULL on STN
2001:105329 GENE TRANSFER TO INTERVERTEBRAL DISC CELLS.
KANG, JAMES D., PITTSBURGH, PA, United States
EVANS, CHRISTOPHER H., PITTSBURGH, PA, United States
NISHIDA, KOTARO, PITTSBURGH, PA, United States
ROBBINS, PAUL D., PITTSBURGH, PA, United States
US 2001006948 A1 20010705
APPLICATION: US 1998-199978 A1 19981125 (9)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 11 OF 13 USPATFULL on STN
2000:149968 Chromatographic purification of adeno-associated virus (AAV).
O'Riordan, Catherine E., Boston, MA, United States
Erickson, Amy E., Charlton, MA, United States
Smith, Alan E., Dover, MA, United States
Genzyme Corporation, Cambridge, MA, United States (U.S. corporation)
US 6143548 20001107
WO 9708298 19970306
APPLICATION: US 1998-11828 19980629 (9)
WO 1996-US13872 19960830 19980629 PCT 371 date 19980629 PCT 102(e) date
PRIORITY: US 1995-2967P 19950830 (60)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 12 OF 13 USPATFULL on STN
1999:99644 Methods and compositions for multiple gene transfer into bone cells.
Bonadio, Jeffrey, Ann Harbor, MI, United States
Goldstein, Steven A., Ann Harbor, MI, United States
The Regent of The University of Michigan, Ann Arbor, MI, United States
(U.S. corporation)
US 5942496 19990824
APPLICATION: US 1994-316650 19940930 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 13 OF 13 USPATFULL on STN
1998:65199 Gene transfer into bone cells and tissues.
Bonadio, Jeffrey, Ann Arbor, MI, United States
Goldstein, Steven A., Ann Arbor, MI, United States
The Regent of the University of Michigan, Ann Arbor, MI, United States
(U.S. corporation)
US 5763416 19980609
APPLICATION: US 1994-199780 19940218 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

=> d l4,cbib,ab,clm,l1

L4 ANSWER 11 OF 13 USPATFULL on STN
2000:149968 Chromatographic purification of adeno-associated virus (AAV).
O'Riordan, Catherine E., Boston, MA, United States
Erickson, Amy E., Charlton, MA, United States
Smith, Alan E., Dover, MA, United States
Genzyme Corporation, Cambridge, MA, United States (U.S. corporation)
US 6143548 20001107
WO 9708298 19970306
APPLICATION: US 1998-11828 19980629 (9)
WO 1996-US13872 19960830 19980629 PCT 371 date 19980629 PCT 102(e) date
PRIORITY: US 1995-2967P 19950830 (60)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to the purification of large scale quantities of active (infectious) **adenovirus** and AAV, especially for use in therapeutic applications. In particular, the invention provides improved methods for contacting such viruses with suitable chromatographic materials in a fashion such that any damage to the virus, particularly to surface components thereof, resulting from contact with such chromatographic materials is minimized or eliminated. The result is the ability to rapidly and efficiently purify commercial level quantities of active (infectious) virus suitable for use in therapeutic applications, e.g. gene transfer/therapy procedures.
CLM What is claimed is:

1. An improved method for purification of adeno-associated virus (AAV) from a composition containing both AAV and **adenovirus**, wherein the improvement comprises inclusion of a step for inactivation and removal of **adenovirus**, said step comprising mixing the composition with a chromatographic matrix material having pores which are approximately the size of **adenovirus**, such that **adenovirus** is inactivated, said chromatographic matrix material being selected from the group consisting of: a. "macroporous" resins, such as macroporous DEAE resins and the BioRad macroporous series (Biorad, Melville, N.Y.); b. heparinized polymers, such as Heparin Agarose (4% cross-linked) (Sigma Chemical, St. Louis, Mo.); c. virus-specific antibodies; such as anti-rep antibodies; d. sulfated resins, such as Sterogene-S (Sulfated Hi Flow) (Sterogene, Carlsbad, Calif.), Spherilose-S (Isco, Lincoln, Nebr.), and Cellufine® sulfate (Amicon, Beverly, Mass.); e. **hydroxyapatite** resins, such as ceramic **hydroxyapatite** resins from Biorad (Melville, N.Y.); f. a series of column separations including the use of ceramic **hydroxyapatite**, DEAE ion-exchange, Cellufine® sulfate, and, optionally, zinc chelate chromatography.

2. The method of claim 1, wherein the chromatographic matrix material comprises a macroporous DEAE resin.

3. The method of claim 1, wherein the chromatographic matrix material comprises a macroporous **hydroxyapatite**.

4. The method of claim 1, wherein the chromatographic matrix material comprises a cellulose or silica membrane resin in conjunction with a macroporous resin.

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=> d his

     (FILE 'HOME' ENTERED AT 18:36:36 ON 22 JUN 2005)

     FILE 'USPATFULL' ENTERED AT 18:36:44 ON 22 JUN 2005
L1          9532 S HYDROXYAPATITE
L2          1599 S L1 AND HYDROXYAPATITE/CLM
L3            69 S L2 AND ADENOVIR?
L4            13 S L3 AND ADENOVIR?/CLM

=> s l3 and ay<2001
       3201823 AY<2001
L5            33 L3 AND AY<2001

=> s l5 not l4
L6            27 L5 NOT L4

=> d l6,ti,1-27

L6   ANSWER 1 OF 27  USPATFULL on STN
TI     Cartilage-Derived morphogenetic proteins

L6   ANSWER 2 OF 27  USPATFULL on STN
TI     Osteogenic devices

L6   ANSWER 3 OF 27  USPATFULL on STN
TI     OSTEOGENIC DEVICES

L6   ANSWER 4 OF 27  USPATFULL on STN
TI     Methods for purifying telomerase

L6   ANSWER 5 OF 27  USPATFULL on STN
TI     Methods for promoting growth of bone using ZVEGF4

L6   ANSWER 6 OF 27  USPATFULL on STN
TI     Bone morphogenetic protein-3 and compositions

L6   ANSWER 7 OF 27  USPATFULL on STN
TI     Therapy for α-galactosidase a deficiency

L6   ANSWER 8 OF 27  USPATFULL on STN
TI     Sustained delivery of polyionic bioactive agents

L6   ANSWER 9 OF 27  USPATFULL on STN
TI     DNA molecules encoding cartilage-derived morphogenetic proteins

L6   ANSWER 10 OF 27  USPATFULL on STN
TI     DNA molecules encoding cartilage-derived morphogenetic proteins
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L6   ANSWER 11 OF 27  USPATFULL on STN
TI     Product and process for T lymphocyte immunosuppression

L6   ANSWER 12 OF 27  USPATFULL on STN
TI     BMP-4 products

L6   ANSWER 13 OF 27  USPATFULL on STN
TI     BMP-3 products

L6   ANSWER 14 OF 27  USPATFULL on STN
TI     Parainfluenza virus glycoproteins and vaccines

L6   ANSWER 15 OF 27  USPATFULL on STN
TI     Product for T lymphocyte immunosuppression

L6   ANSWER 16 OF 27  USPATFULL on STN
TI     In vivo gene transfer methods for wound healing

L6   ANSWER 17 OF 27  USPATFULL on STN
TI     Devices comprising chondrogenic protein and methods of inducing
       endochondral bone formation therewith

L6   ANSWER 18 OF 27  USPATFULL on STN
TI     Formation of human bone in vivo using ceramic powder and human marrow
       stromal fibroblasts

L6   ANSWER 19 OF 27  USPATFULL on STN
TI     BMP-15 compositions

L6   ANSWER 20 OF 27  USPATFULL on STN
TI     Neutrophil inhibitors

L6   ANSWER 21 OF 27  USPATFULL on STN
TI     Bone morphogenetic protein-10 (BMP-10) compositions

L6   ANSWER 22 OF 27  USPATFULL on STN
TI     Bone morphogenetic protein-9 compositions

L6   ANSWER 23 OF 27  USPATFULL on STN
TI     Compositions comprising bone morphogenetic protein-2 (BMP-2)

L6   ANSWER 24 OF 27  USPATFULL on STN
TI     Purified thermostable nucleic acid polymerase enzyme from Thermotoga
       maritima

L6   ANSWER 25 OF 27  USPATFULL on STN
TI     Bone morphogenetic protein 5(BMP-5) compositions

L6   ANSWER 26 OF 27  USPATFULL on STN
TI     Process of preparing a soluble LDL receptor

L6   ANSWER 27 OF 27  USPATFULL on STN
TI     Osteogenic devices


=> d his

     (FILE 'HOME' ENTERED AT 18:36:36 ON 22 JUN 2005)

     FILE 'USPATFULL' ENTERED AT 18:36:44 ON 22 JUN 2005
L1          9532 S HYDROXYAPATITE
L2          1599 S L1 AND HYDROXYAPATITE/CLM
L3            69 S L2 AND ADENOVIR?
L4            13 S L3 AND ADENOVIR?/CLM
L5            33 S L3 AND AY<2001
L6            27 S L5 NOT L4

=> s us5496926/pn
L7             1 US5496926/PN

=> d l7,cbib,clm

L7   ANSWER 1 OF 1  USPATFULL on STN
96:19199 Process of preparing a soluble LDL receptor.
    Rubinstein, Menachem, Givat Shmuel, Israel
    Novick, Daniela, Rehovot, Israel
    Tal, Nathan, Rehovot, Israel
    Yeda Research and Development Co. Ltd., Rehovot, Israel (non-U.S.
```

corporation)
US 5496926 19960305 <--
APPLICATION: US 1993-92817 19930719 (8)
PRIORITY: IL 1992-100696 19920119
IL 1992-102915 19920823
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:

1. A process for the preparation of a soluble LDL receptor protein, comprising: (a) treating, with interferon-γ, cells capable of entering an antiviral state in response to induction by interferon-γ to produce soluble LDL receptor protein; (b) isolating the soluble LDL receptor protein from the supernatant; and (c) purifying the soluble LDL receptor.

2. A process in accordance with claim 1, wherein said cells are mammalian cells.

3. A process in accordance with claim 1, wherein said cells are human cells and said soluble LDL receptor protein has a molecular weight of 28 kD when measured by SDS-PAGE under reducing conditions, and includes the amino acid sequence of SEQ ID NO:6.

4. A process according to claim 1, wherein said cells are human WISH cells.

5. A process for preparing a soluble LDL receptor protein, comprising: (a) growing to confluency cells capable of entering an antiviral state in response to induction by interferon-γ to produce soluble LDL receptor protein; (b) inducing the cells with interferon-γ; (c) harvesting the culture supernatant; (d) concentrating the supernatant; (e) subjecting the concentrated supernatant of step (d) to anion exchange chromatography and selecting the fraction having antiviral activity; (f) applying the fraction obtained in step (e) to chromatography on a hydroxyapatite column and selecting the fraction having antiviral activity; (g) applying the fraction obtained in step (f) to anion HPLC and selecting the fraction having antiviral activity; (h) applying the fraction obtained in step (g) to hydrophobic interaction chromatography and selecting the fraction having antiviral activity; (i) applying the fraction obtained in step (h) to reverse phase HPLC and selecting the fraction having antiviral activity; and (j) repeating step (i) to obtain soluble LDL receptor purified to homogeneity.

6. A process according to claim 5, wherein at least one of the chromatography steps (e)-(j) is replaced by immunoaffinity chromatography on an anti LDL receptor monoclonal antibody column, the monoclonal antibody of the monoclonal antibody column having an epitope binding region specific for an epitope of said soluble LDL receptor protein.

7. A process according to claim 6, wherein the monoclonal antibody is C7 (ATCC, CRL 1691) and the soluble receptor is eluted at a high pH.

8. A process according to claim 5, wherein said cells are human WISH cells.

9. A process in accordance with claim 5, wherein said cells are mammalian cells.

10. A process in accordance with claim 5, wherein said cells are human cells and said soluble LDL receptor protein has a molecular weight of 28 kD when measured by SDS-PAGE under reducing conditions, and includes the amino acid sequence of SEQ ID NO:6.

11. A process for preparing a soluble LDL receptor protein from a biological fluid sample isolated from a mammalian body, comprising: (a) concentrating the biological fluid sample; (b) subjecting the concentrated sample of step (b) to anion exchange chromatography and selecting the fraction having antiviral activity; (c) applying the fraction obtained in step (b) to chromatography on a hydroxyapatite column and selecting the fraction having antiviral activity; (d) applying the fraction obtained in step (c) to anion HPLC and selecting the fraction having antiviral activity; (e) applying the fraction obtained in step (d) to hydrophobic interaction chromatography and selecting the fraction having antiviral activity; (f) applying the fraction obtained in step (e) to reverse phase HPLC and selecting the fraction having antiviral activity; (g) repeating step (f) to obtain

soluble LDL receptor purified to homogeneity.

12. A process according to claim 11, wherein at least one of the steps (b)-(g) is replaced by immunoaffinity chromatography on an anti-LDL receptor monoclonal antibody column, said monoclonal antibody of the monoclonal antibody column having an epitope binding region specific for an epitope of said soluble LDL receptor protein.

13. A process according to claim 12, wherein the monoclonal antibody is C7 (ATCC, CRL 1691) and the soluble receptor is eluted at a high pH.

14. A process for obtaining a soluble LDL receptor protein, comprising: obtaining a biological fluid sample from a mammalian body; and isolating the soluble LDL receptor protein therefrom.

=> d his

(FILE 'HOME' ENTERED AT 18:36:36 ON 22 JUN 2005)

FILE 'USPATFULL' ENTERED AT 18:36:44 ON 22 JUN 2005
L1 9532 S HYDROXYAPATITE
L2 1599 S L1 AND HYDROXYAPATITE/CLM
L3 69 S L2 AND ADENOVIR?
L4 13 S L3 AND ADENOVIR?/CLM
L5 33 S L3 AND AY<2001
L6 27 S L5 NOT L4
L7 1 S US5496926/PN

=> s l7 and adenovir?
33013 ADENOVIR?
L8 1 L7 AND ADENOVIR?

=> d l8,kwic

L8 ANSWER 1 OF 1 USPATFULL on STN
PI US 5496926 19960305 <--
DETD . . . sequences may be employed, depending on the nature of the host. They may be derived from viral sources, such as **adenovirus**, bovine papilloma virus, Simian virus, or the like, where the regulatory signals are associated with a particular gene which has. . .

=> s us5624833/pn
L9 1 US5624833/PN

=> d l9,cbib,clm

L9 ANSWER 1 OF 1 USPATFULL on STN
97:36094 Purified thermostable nucleic acid polymerase enzyme from Thermotoga maritima.
Gelfand, David H., Oakland, CA, United States
Lawyer, Frances C., Oakland, CA, United States
Stoffel, Susanne, El Cerrito, CA, United States
Hoffmann-La Roche Inc., Nutley, NJ, United States (U.S. corporation)
US 5624833 19970429 <--
APPLICATION: US 1995-475231 19950607 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:
1. A purified thermostable DNA polymerase I enzyme that catalyzes combination of nucleoside triphosphates to form a nucleic acid strand complementary to a nucleic acid template strand, said enzyme derived from the eubacterium Thermotoga maritima.

2. The enzyme of claim 1 having a molecular weight of about 97 kilodaltons as determined by SDS-page, a predicted molecular weight of about 102 kilodaltons.

3. The enzyme of claim 1 that has reverse transcriptase activity.

4. The enzyme of claim 1 that has 3'→5' exonuclease activity.

5. The enzyme of claim 1 that is in native form.

6. The enzyme of claim 2 that is in native form.

7. The enzyme of claim 1 that is in recombinant form.

8. The enzyme of claim 2 that is in recombinant form.

9. The enzyme of claim 2 that has reverse transcriptase and 3'→5' exonuclease activity.

10. The enzyme of claim 9 that is in native form.

11. The enzyme of claim 9 that is in recombinant form.

12. A method for purifying Thermotoga maritima DNA polymerase from T. maritima cells, said method comprising the steps of: (a) preparing a crude cell extract from said cells; (b) adjusting the ionic strength of said extract so that said polymerase dissociates from any nucleic acid in said extract; (c) subjecting the extract to hydrophobic interaction chromatography; (d) subjecting the extract to DNA binding protein affinity chromatography; (e) subjecting the extract to nucleotide binding protein affinity chromatography; and (f) subjecting the extract to chromatography selected from the group consisting of anion exchange, cation exchange, and hydroxyapatite chromatography.

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=> d his

     (FILE 'HOME' ENTERED AT 18:36:36 ON 22 JUN 2005)

     FILE 'USPATFULL' ENTERED AT 18:36:44 ON 22 JUN 2005
L1          9532 S HYDROXYAPATITE
L2          1599 S L1 AND HYDROXYAPATITE/CLM
L3            69 S L2 AND ADENOVIR?
L4            13 S L3 AND ADENOVIR?/CLM
L5            33 S L3 AND AY<2001
L6            27 S L5 NOT L4
L7             1 S US5496926/PN
L8             1 S L7 AND ADENOVIR?
L9             1 S US5624833/PN

=> s l9 and adenovir?
         33013 ADENOVIR?
L10          1 L9 AND ADENOVIR?

=> d l10,kwic

L10  ANSWER 1 OF 1  USPATFULL on STN
PI     US 5624833              19970429                              <--
SUMM   . . . 40 (SV 40) (Fiers et al., 1978, Nature 273: 113), or other
       viral promoters such as those derived from polyoma, adenovirus 2,
       bovine papilloma virus (BPV), or avian sarcoma viruses, or
       immunoglobulin promoters and heat shock promoters. A system for
       expressing. . .


=> e erickson amy e/in
E1            1     ERICKSON ALVE JOHN/IN
E2            1     ERICKSON ALVIN L/IN
E3            1 --> ERICKSON AMY E/IN
E4            1     ERICKSON ANDREW IVAR/IN
E5            2     ERICKSON ANDREW N/IN
E6            3     ERICKSON ANDREW NORMAN/IN
E7            2     ERICKSON ANITA/IN
E8            1     ERICKSON ANITA S/IN
E9            2     ERICKSON ANN HART/IN
E10           6     ERICKSON ARLEN J/IN
E11           1     ERICKSON ARLEN JEROME/IN
E12           5     ERICKSON ARNOLD R/IN

=> s e3
L11           1 "ERICKSON AMY E"/IN

=> d l11,cbib

L11  ANSWER 1 OF 1  USPATFULL on STN
2000:149968 Chromatographic purification of adeno-associated virus (AAV).
    O'Riordan, Catherine E., Boston, MA, United States
    Erickson, Amy E., Charlton, MA, United States
    Smith, Alan E., Dover, MA, United States
    Genzyme Corporation, Cambridge, MA, United States (U.S. corporation)
    US 6143548 20001107
```

WO 9708298 19970306
APPLICATION: US 1998-11828 19980629 (9)
WO 1996-US13872 19960830 19980629 PCT 371 date 19980629 PCT 102(e) date
PRIORITY: US 1995-2967P 19950830 (60)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

=> e shabram paul w/in
E1 1 SHABRAM PATRICT L/IN
E2 3 SHABRAM PAUL/IN
E3 5 --> SHABRAM PAUL W/IN
E4 3 SHABRAM SR LYLE F/IN
E5 6 SHABRANG MANI/IN
E6 2 SHABSIGH RIDWAN/IN
E7 1 SHABTAI BEN ZION/IN
E8 11 SHABTAI JOSEPH/IN
E9 8 SHABTAI JOSEPH S/IN
E10 1 SHABTAI LIOR/IN
E11 1 SHABTAI MORDECHAI/IN
E12 1 SHABTAI MOTI/IN

=> s e3
L12 5 "SHABRAM PAUL W"/IN

=> d l12,cbib,1-5

L12 ANSWER 1 OF 5 USPATFULL on STN
2002:329464 Methods and compositions for reducing immune response.
LaFace, Drake M., San Diego, CA, UNITED STATES
Rahman, Amena, San Diego, CA, UNITED STATES
Shabram, Paul W., Olivenhain, CA, UNITED STATES
Tsai, Van T., San Diego, CA, UNITED STATES
US 2002187143 A1 20021212
APPLICATION: US 2002-222722 A1 20020816 (10)
PRIORITY: US 1999-152650P 19990907 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L12 ANSWER 2 OF 5 USPATFULL on STN
2002:272442 Selectively replicating viral vectors.
Ramachandra, Muralidhara, San Diego, CA, UNITED STATES
Shabram, Paul W., Olivenhain, CA, UNITED STATES
US 2002150557 A1 20021017
APPLICATION: US 2002-62216 A1 20020130 (10)
PRIORITY: US 1998-104399P 19981015 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L12 ANSWER 3 OF 5 USPATFULL on STN
2002:268409 Methods and compositions for reducing immune response.
LaFace, Drake M., San Diego, CA, United States
Rahman, Amena, San Diego, CA, United States
Shabram, Paul W., Olivenhain, CA, United States
Tsai, Van T., San Diego, CA, United States
Canji, Inc., San Diego, CA, United States (U.S. corporation)
US 6464976 B1 20021015
APPLICATION: US 2000-653474 20000831 (9)
PRIORITY: US 1999-152650P 19990907 (60)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L12 ANSWER 4 OF 5 USPATFULL on STN
1999:155518 Viral production process.
Giroux, Daniel D., La Jolla, CA, United States
Goudreau, Ann M., San Diego, CA, United States
Ramachandra, Muralidhara, San Diego, CA, United States
Shabram, Paul W., Olivenhain, CA, United States
Canji, Inc., San Diego, CA, United States (U.S. corporation)
US 5994134 19991130
APPLICATION: US 1998-73076 19980504 (9)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L12 ANSWER 5 OF 5 USPATFULL on STN
1998:143924 Method of purification of viral vectors.
Shabram, Paul W., Olivenhain, CA, United States
Huyghe, Bernard G., San Diego, CA, United States
Liu, Xiaodong, New York, NY, United States

Shepard, H. Michael, Rancho Santa Fe, CA, United States
Canji, Inc., San Diego, CA, United States (U.S. corporation)
US 5837520 19981117
APPLICATION: US 1995-400793 19950307 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

=> d l12,cbib,clm,4,5

L12 ANSWER 4 OF 5 USPATFULL on STN
1999:155518 Viral production process.
Giroux, Daniel D., La Jolla, CA, United States
Goudreau, Ann M., San Diego, CA, United States
Ramachandra, Muralidhara, San Diego, CA, United States
Shabram, Paul W., Olivenhain, CA, United States
Canji, Inc., San Diego, CA, United States (U.S. corporation)
US 5994134 19991130
APPLICATION: US 1998-73076 19980504 (9)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:

1. A method of achieving a cell density greater than 5×10^6 producer cells/ml in a microcarrier based bioreactor process for the production of a virus in a producer cell, said method comprising the steps of: a) preparing a culture of producer cells attached to microcarriers wherein the ratio of producer cells to microcarriers is approximately 10 cells/microcarrier b) seeding the bioreactor with a quantity of the producer cell-coated microcarriers prepared in step (a) to a density greater than approximately 6 grams (based on the dry weight of the microcarrier) of producer cell-coated microcarriers per liter of bioreactor media volume; and c) culturing the producer cells in the bioreactor under perfusion conditions in serum containing media to a density of greater than 100 cells/microcarrier.

2. The method of claim 1, wherein the producer cell is a 293 cell.

3. The method of claim 2 wherein the virus is an adenovirus.

4. The method of claim 3 wherein the virus is a replication defective adenovirus derived from the adenovirus type 5 genome.

5. The method of claim 4 wherein the replication defective adenovirus further comprises an expression cassette for an exogenous transgene.

6. The method of claim 5 wherein the exogenous transgene is selected from the group consisting of tumor suppressor genes, cytotoxic genes, cytostatic genes, proapoptotic genes, or prodrug activating genes.

7. The method of claim 6 wherein the exogenous transgene is a tumor suppressor gene.

8. The method of claim 7 wherein the tumor suppressor gene is p53.

9. A method producing a population of producer cells containing a high titer of viral particles in a microcarrier based bioreactor in serum free media, said method comprising the process of claim 1 further comprising the steps of: d) removing the serum containing medium; e) synchronizing the producer cells in G1 phase; f) infecting the producer cells with a virus; g) culturing cells under conditions to permit viral replication until a maximum point is achieved.

10. The method of claim 9, wherein the producer cell is a 293 cell.

11. The method of claim 10 wherein cell synchronization is achieved by holding the cells in a non-serum medium for greater than approximately one-third of a cell cycle.

12. The method of claim 11 wherein the virus is an adenovirus.

13. The method of claim 12 wherein the virus is a replication defective adenovirus derived from the adenovirus type 5 genome.

14. The method of claim 13 wherein the replication defective adenovirus further comprises an expression cassette for an exogenous transgene.

15. The method of claim 14 wherein the exogenous transgene is selected from the group consisting of tumor suppressor genes, cytotoxic genes, cytostatic genes, proapoptotic genes, or prodrug activating genes.

16. The method of claim 9, further comprising the steps of: h) harvesting the cells; i) lysing the producer cells; j) isolating the viral particles from the cell lysate; and k) purifying the intact viral particles.

17. The method of claim 11 wherein the virus is an adenovirus.

L12 ANSWER 5 OF 5 USPATFULL on STN
1998:143924 Method of purification of viral vectors.
Shabram, Paul W., Olivenhain, CA, United States
Huyghe, Bernard G., San Diego, CA, United States
Liu, Xiaodong, New York, NY, United States
Shepard, H. Michael, Rancho Santa Fe, CA, United States
Canji, Inc., San Diego, CA, United States (U.S. corporation)
US 5837520 19981117
APPLICATION: US 1995-400793 19950307 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed:
1. A method for purification of an intact viral particle from a cell lysate, the method comprising: a) treating said cell lysate which contains said intact viral particle with an enzymatic agent that selectively degrades both unencapsulated DNA and RNA; b) chromatographing the treated lysate from step a) on a first resin; and c) chromatographing the eluant from step b) on a second resin; wherein one resin is an anion exchange resin and the other is an immobilized metal ion affinity resin.

2. The method of claim 1, wherein the first resin is an anion exchange resin and the second resin is an immobilized metal affinity resin.

3. The method of claim 1, which comprises the additional step of filtering the treated lysate from step (a).

4. The method of claim 1, which comprises the additional step of buffering the pH of the cell lysate between about 5.0 and about 9.0 before applying it to the first resin.

5. The method of claim 1, wherein the viral particle is a retrovirus.

6. The method of claim 1, wherein the viral particle is an adenovirus.

7. The method of claim 6, wherein the adenoviral particle is a recombinant viral particle which comprises a tumor suppressor gene.

8. The method of claim 1, wherein the adenoviral particle is a type 2 or type 5 adenovirus.

9. The method of claim 8, wherein the adenoviral particle is a type 5 adenovirus.

10. The method of claim 7, wherein the tumor suppressor gene is a wild-type p53 gene.

11. The method of claim 1, wherein the anion exchange resin is chosen from the group consisting of DEAE, TMAE, DMAE, QAE and PEI.

12. The method of claim 11, wherein the anion exchange resin is DEAE resin.

13. The method of claim 1, wherein the immobilized metal affinity resin is charged with a divalent cation of a metal chosen from the group consisting of cobalt, nickel, copper, and zinc.

14. The method of claim 13, wherein the immobilized metal affinity resin is a TED or an IDA resin.

15. The method of claim 14, wherein the immobilized metal affinity resin is an IDA-(cross-linked agarose) resin.

16. The method of claim 13, wherein the divalent cation is zinc.

17. The method of claim 1, wherein the enzymatic agent that selectively degrades both unencapsulated DNA and RNA is one or more enzymes.

18. The method of claim 17, wherein the one or more enzymes are endonucleases.

19. The method of claim 18, wherein the enzyme is a mixture of RNase and DNase.

20. A method for purification of intact viral particles from a cell lysate, the method comprising the steps of: a) treating said cell lysate which contains said intact viral particle with an enzymatic agent that selectively degrades both unencapsulated DNA and RNA; b) chromatographing the treated lysate from step a) on a first resin, and c) chromatographing the eluant from step b) on a second resin; wherein: one resin is an anion exchange resin and the other is a hydrophobic interaction chromatography resin; or one resin is a cation exchange resin and the other is either a hydrophobic interaction chromatography resin or an immobilized metal ion affinity resin.

21. The method of claim 20, wherein one resin is an anion exchange resin and the other is a hydrophobic interaction chromatography resin.

22. The method of claim 21, wherein the first resin is an anion exchange resin and the second is a hydrophobic interaction chromatography resin.

23. The method of claim 20, wherein one resin is a cation exchange resin and the other is an immobilized metal ion affinity resin.

24. The method of claim 20, wherein one resin is a cation exchange resin and the other is a hydrophobic interaction chromatography resin.

25. A method of determining the number of intact viral particles in a sample, the method comprising: a) chromatographing the sample containing the intact viral particles on an anion exchange resin; b) monitoring the absorbance of the eluate from the chromatography of step (a) at a selected wavelength; and c) determining the total number of intact viral particles in the sample by comparing the absorbance value obtained in step (b) to a standard curve which relates absorbance to number of viral particles.

26. The method of claim 25, wherein the anion exchange resin has QAE groups covalently attached to a polystyrene/divinyl benzene copolymer support.

27. The method of claim 25, wherein the method further comprises chromatographing the eluate from the chromatography of step (a) on a second resin, wherein the second resin is an immobilized metal ion affinity resin.

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=> d his

     (FILE 'HOME' ENTERED AT 18:36:36 ON 22 JUN 2005)

     FILE 'USPATFULL' ENTERED AT 18:36:44 ON 22 JUN 2005
L1          9532 S HYDROXYAPATITE
L2          1599 S L1 AND HYDROXYAPATITE/CLM
L3            69 S L2 AND ADENOVIR?
L4            13 S L3 AND ADENOVIR?/CLM
L5            33 S L3 AND AY<2001
L6            27 S L5 NOT L4
L7             1 S US5496926/PN
L8             1 S L7 AND ADENOVIR?
L9             1 S US5624833/PN
L10            1 S L9 AND ADENOVIR?
               E ERICKSON AMY E/IN
L11            1 S E3
               E SHABRAM PAUL W/IN
L12            5 S E3

=> file wpids
COST IN U.S. DOLLARS                          SINCE FILE      TOTAL
                                                 ENTRY    SESSION
FULL ESTIMATED COST                              51.20      51.41

FILE 'WPIDS' ENTERED AT 18:46:16 ON 22 JUN 2005
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MOST RECENT DERWENT UPDATE:     200539        <200539/DW>
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    GUIDES, PLEASE VISIT:
    http://thomsonderwent.com/support/userguides/              <<<

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    DOCUMENTATION NOW AVAILABLE IN DERWENT WORLD PATENTS INDEX
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    FOR FURTHER DETAILS: http://www.thomsonderwent.com/dwpifv  <<<

>>> THE CPI AND EPI MANUAL CODES HAVE BEEN REVISED FROM UPDATE 200501.
    PLEASE CHECK:
http://thomsonderwent.com/support/dwpiref/reftools/classification/code-revision/
    FOR DETAILS. <<<

=> s hydroxyapatite
L13       2918 HYDROXYAPATITE

=> s l13 and adenovir?
          3801 ADENOVIR?
L14         14 L13 AND ADENOVIR?

=> s l14 and py<2001


SEARCH ENDED BY USER

=>
SEARCH ENDED BY USER

=> s l14 and py<2001


SEARCH ENDED BY USER

=> s l14 and py<2001


SEARCH ENDED BY USER

=> d l14,bib,ab,1-14

L14  ANSWER 1 OF 14  WPIDS  COPYRIGHT 2005 THE THOMSON CORP on STN
Full Text
AN   2005-331961 [34]   WPIDS
CR   1999-590379 [50]; 2001-450477 [48]; 2003-465598 [44]; 2003-742824 [70];
     2003-811733 [76]
DNC  C2005-103147
TI   Preparation of a compound having telomerase regulating activity, used to
     treat e.g. cancer, comprises combining obtained mammalian telomerase
     enzyme preparation with test compound; and determining and identifying the
     telomerase activity.
DC   B04 B05 D16
IN   ATKINSON, E M; LICHTSTEINER, S P; PRUZAN, R A; VASSEROT, A P; WEINRICH, S
     L
PA   (ATKI-I) ATKINSON E M; (LICH-I) LICHTSTEINER S P; (PRUZ-I) PRUZAN R A;
     (VASS-I) VASSEROT A P; (WEIN-I) WEINRICH S L
CYC  1
PI   US 2005089883   A1 20050428 (200534)*      24
ADT  US 2005089883 A1 CIP of US 1995-510736 19950804, Cont of US 1997-833377
     19970404, CIP of US 1999-420056 19991018, Cont of US 2000-717828 20001120,
     Cont of US 2002-330872 20021224, US 2004-811033 20040326
FDT  US 2005089883 A1 Cont of US 5968506, CIP of US 6261556, Cont of US
     6517834, Cont of US 6787133
PRAI US 2000-717828       20001120; US 1995-510736       19950804;
     US 1997-833377       19970404; US 1999-420056       19991018;
     US 2002-330872       20021224; US 2004-811033       20040326
AB   US2005089883 A UPAB: 20050527
     NOVELTY - Production of a compound (I) that regulates telomerase activity
     comprising obtaining a mammalian telomerase enzyme preparation (A);
     combining (A) with a test compound; determining telomerase activity of the
     enzyme in the presence of the test compound; identifying (I) as being a
     telomerase regulator if the telomerase activity measured is affected by
```

the presence of (I); and then producing (I), is new.

DETAILED DESCRIPTION - Production of a compound (I) that regulates telomerase activity comprises obtaining a mammalian telomerase enzyme preparation (A) (at least approximately 2000-fold more pure than an extract of cells from **adenovirus**-transformed kidney cell line (293 cells) and where the telomerase enzyme contains telomerase RNA component and has a molecular weight of 200-2000 kDa); combining (A) with a test compound; determining telomerase activity of the enzyme in the presence of the test compound; identifying (I) as being a telomerase regulator if the telomerase activity measured is affected by the presence of (I); and then producing (I) if it is identified as being a telomerase regulator.

ACTIVITY - Cytostatic.

No biological data given.

MECHANISM OF ACTION - Telomerase regulator; Telomerase inhibitor; Telomerase activator.

USE - (I) is useful for the treatment of age-related diseases and diseases associated with it, including cancer.

ADVANTAGE - The method provides telomerase preparations with high purity of at least 3000-fold increased relative purity, 20000-fold increased relative purity, 60000-fold increased relative purity or 100000-fold increased relative purity.

Dwg.0/7

L14 ANSWER 2 OF 14 WPIDS COPYRIGHT 2005 THE THOMSON CORP on STN
Full Text
AN 2005-322638 [33] WPIDS
DNN N2005-263930 DNC C2005-100583
TI Implant for regenerating bone or cartilage, comprises biocompatible material containing **adenoviral** vector with transcriptional factor gene of bone/cartilage inductive transcription factor or retroviral vector.
DC A96 B04 D16 D22 P34
IN KOJIMA, H; UEMURA, T
PA (NISC-N) JAPAN SCI & TECHNOLOGY AGENCY; (NAAD-N) NAT INST ADVANCED IND SCI & TECHNOLOGY
CYC 108
PI WO 2005035014 A1 20050421 (200533)* JA 42
RW: AT BE BG BW CH CY CZ DE DK EA EE ES FI FR GB GH GM GR HU IE IT KE LS LU MC MW MZ NA NL OA PL PT RO SD SE SI SK SL SZ TR TZ UG ZM ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BW BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE EG ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NA NI NO NZ OM PG PH PL PT RO RU SC SD SE SG SK SL SY TJ TM TN TR TT TZ UA UG US UZ VC VN YU ZA ZM ZW
ADT WO 2005035014 A1 WO 2004-JP15673 20041015
PRAI JP 2003-355505 20031015
AB WO2005035014 A UPAB: 20050524
NOVELTY - A bone or cartilage regenerating implant comprising biocompatible material containing **adenoviral** vector with transcriptional factor gene of bone/cartilage inductive transcription factor or retroviral vector, is new.

USE - For regenerating bone or cartilage.

ADVANTAGE - The implant enables favorable degeneration of bone or cartilage by releasing the transcriptional factor in sustained manner. The bone/cartilage regeneration can be performed conveniently and safely.

DESCRIPTION OF DRAWING(S) - The figure shows the diagram of rat femur defect portion replanting experiment.

Dwg.3/12

L14 ANSWER 3 OF 14 WPIDS COPYRIGHT 2005 THE THOMSON CORP on STN
Full Text
AN 2005-161610 [17] WPIDS
CR 2000-687541 [67]; 2001-611088 [70]; 2002-573696 [61]; 2003-352153 [33]; 2003-874621 [81]; 2004-070738 [07]; 2004-225671 [21]; 2005-011673 [01]
DNC C2005-052146
TI Promoting growth of bone, ligament, or cartilage in mammal comprises administering a composition comprising z-vascular endothelial growth factor-4 (zvegf4) in combination with delivery vehicle to a mammal.
DC A96 B04 D16 D21 D22
IN GILBERTSON, D G; HART, C E
PA (GILB-I) GILBERTSON D G; (HART-I) HART C E
CYC 1
PI US 2005031694 A1 20050210 (200517)* 31
ADT US 2005031694 A1 Provisional US 1999-132250P 19990503, Provisional US 1999-164463P 19991110, Provisional US 2000-180169P 20000204, Div ex US 2000-540224 20000331, Cont of US 2002-226559 20020823, US 2004-910938 20040803
FDT US 2005031694 A1 Div ex US 6468543
PRAI US 2004-910938 20040803; US 1999-132250P 19990503;
US 1999-164463P 19991110; US 2000-180169P 20000204;

US 2000-540224 20000331; US 2002-226559 20020823

AB US2005031694 A UPAB: 20050311

NOVELTY - Promoting (M1) growth of bone, ligament, or cartilage in a mammal comprising administering a composition comprising a z-vascular endothelial growth factor-4 (zvegf4) in combination with a delivery vehicle to the mammal is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for:

(1) promoting (M2) proliferation or differentiation of cells, comprises culturing the cells in zvegf4, where the cells are osteoblasts, osteoclasts, chondrocytes, or bone marrow stem cells; and

(2) promoting (M3) cartilage growth, comprising culturing chondrocytes ex vivo in the presence of zvegf4 under conditions, where the chondrocytes proliferate, and placing the cultured chondrocytes into a mammal, where cartilage is to be grown.

ACTIVITY - Osteopathic.

MECHANISM OF ACTION - Promotes proliferation or differentiation of cells (claimed).

The ability of zvegf4 to promote proliferation of cells was determined in vivo. Mice (C57BL6) were infected with a zvegf4-encoding **adenovirus** vector (AdZyvegf4) to determine the effects on serum chemistry, complete blood counts (CBC), body and organ weight changes, and histology. Group 1 mice received green fluorescent protein (green fluorescent protein (GFP) control **adenovirus**. Group 2 mice received zvegf4 **adenovirus**. Group 3 mice were untreated controls. The mice received injections of the appropriate **adenovirus** solution. Blood was collected for CBCs and clinical chemistry measurements. On day 20, mice were weighed and sacrificed by cervical dislocation. Tissues were collected for histopathology. Spleen and liver weight was significantly greater in all AdZyvegf4-treated mice. In the liver, proliferation of sinusoidal endothelial cells were observed. In the spleen, proliferation of reticuloendothelial cells were observed.

USE - (M1) is useful for promoting growth of bone, ligament, or cartilage in a mammal (claimed).

Dwg.0/1

L14 ANSWER 4 OF 14 WPIDS COPYRIGHT 2005 THE THOMSON CORP on STN

Full Text

AN 2004-737348 [72] WPIDS

DNN N2004-583520 DNC C2004-259277

TI Three-dimensional construct, for presenting biological information to cell or tissue, has polymeric matrix, and nanoparticle coated with monomolecular layer with biological information, dispersed in polymeric matrix.

DC A89 B04 D16 S03

IN CHEN, I; SHASTRI, V P; ZNIDARSIC, W

PA (CHIL-N) CHILDRENS HOSPITAL PHILADELPHIA; (UYPE-N) UNIV PENNSYLVANIA

CYC 108

PI WO 2004085998 A2 20041007 (200472)* EN 38

RW: AT BE BG BW CH CY CZ DE DK EA EE ES FI FR GB GH GM GR HU IE IT KE LS LU MC MW MZ NL OA PL PT RO SD SE SI SK SL SZ TR TZ UG ZM ZW

W: AE AG AL AM AT AU AZ BA BB BG BR BW BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE EG ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NA NI NO NZ OM PG PH PL PT RO RU SC SD SE SG SK SL SY TJ TM TN TR TT TZ UA UG US UZ VC VN YU ZA ZM ZW

ADT WO 2004085998 A2 WO 2004-US9192 20040326

PRAI US 2003-458258P 20030328

AB WO2004085998 A UPAB: 20041109

NOVELTY - A three-dimensional construct (I), comprising a polymeric matrix, and a nanoparticle having a structure and a chemical functional group attached to the structure, where the nanoparticle has a diameter of 5 nm-10 microns and is coated with a monomolecular layer comprising biological information and dispersed in the polymeric matrix at a density of 0.01 vol%, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) preparing (M2) (I), comprising:

(a) providing the polymeric matrix, providing an unprocessed nanoparticle;

(b) making the nanoparticle by contacting the unprocessed nanoparticle with a carrier of biological information to form the monomolecular layer; and

(c) dispersing the nanoparticle in the polymeric matrix at the density of 0.01 vol% and thus making (I);

(2) a nanoparticle (II) comprising a structure chosen from silicon oxide functionalized with a chemical functional group, (poly)lactic acid, (poly)lactic-co-glycolic acid and (poly)anhydride, a monomolecular layer of **hydroxyapatite**, and optionally a monomolecular layer of (poly)acrylic acid and/or a monomolecular layer of collagen, where the structure is

coated with the monomolecular layer of **hydroxyapatite** and optionally with the monomolecular layer of (poly)acrylic acid and/or monomolecular layer of collagen, provided that the monomolecular layer of **hydroxyapatite** is an outermost monomolecular layer; and

(3) administering (II) to a cell, comprising providing (II), optionally providing an auxiliary surface chosen from polymer, carbonaceous material, wool, glass, ceramic and metal, where the auxiliary surface is in communication with (II), and contacting the cell with (II) and thus administering (II).

USE - (I) is useful for presenting biological information to a cell or a tissue, which involves providing (I) and contacting (I) with the cell or the tissue to present the biological information and thus affecting at least one characteristic of the cell or the tissue. The diameter, the biological information and the density are selected to affect at least one characteristic of the cell or the tissue. The biological information includes a biomolecule, polymer and bone substitute. The characteristic of the cell or tissue is proliferation or differentiation. The monomolecular layer comprises at least one of a monomolecular layer of poly(acrylic acid) and a monomolecular layer of collagen as the intermediate monomolecular layer, provided that the monomolecular layer comprising **hydroxyapatite** is the outermost monomolecular layer. The **hydroxyapatite** is deposited onto collagen from an aqueous mixture comprising calcium nitrate tetrahydrate and ammonium phosphate at a molar ratio of 1.5-2 and pH of 7-9.5, preferably the molar ratio is equal to 2. The method further involves contacting (I) with an auxiliary surface prior to contacting (I) with the cell or tissue. The auxiliary surface is chosen from polymer, carbonaceous material, wool, glass, ceramic and metal. The auxiliary surface is in a shape of a mesh, fiber, sheet, sponge, layer, pattern and pre-formed object. (All claimed.) (I) has wide-ranging applicability in areas of tissue engineering, medical devices, medical implants, bio-micro electro-mechanical systems and high throughput screening technologies.

Dwg.0/11

L14 ANSWER 5 OF 14 WPIDS COPYRIGHT 2005 THE THOMSON CORP on STN
Full Text
AN 2003-788077 [74] WPIDS
DNC C2003-217499
TI Implant used as bone substitute for regeneration comprises biocompatible material with bone marrow originated cells having growth factor gene transferred into them.
DC A96 B04 D16
IN KOJIMA, H; MATSUMOTO, K; TATEISHI, T; UEMURA, T
PA (NISC-N) JAPAN SCI & TECHNOLOGY CORP; (NAAD-N) NAT INST ADVANCED IND SCI & TECHNOLOGY
CYC 3
PI WO 2003070291 A1 20030828 (200374)* JA 37
W: CA JP US
JP 2003569244 X 20050609 (200538) 20
ADT WO 2003070291 A1 WO 2002-JP10866 20021021; JP 2003569244 X WO 2002-JP10866 20021021, JP 2003-569244 20021021
FDT JP 2003569244 X Based on WO 2003070291
PRAI JP 2002-41604 20020219
AB WO2003070291 A UPAB: 20031117
NOVELTY - Implant comprises a biocompatible material containing cells transferred with a growth factor gene.

DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for production of the implant which comprises in vitro differentiation induction of bone marrow originated cells into osteoblasts, transfecting the cells with a growth factor gene, and disseminating a biocompatible material with the resultant cells.

ACTIVITY - Osteopathic.

MECHANISM OF ACTION - None given.

USE - The implant is used as bone substitute for regeneration.

ADVANTAGE - The implant is highly biocompatible and enables rapid bone regeneration.

Dwg.0/10

L14 ANSWER 6 OF 14 WPIDS COPYRIGHT 2005 THE THOMSON CORP on STN
Full Text
AN 2003-430202 [40] WPIDS
DNC C2003-113633
TI Transferring nucleic acid into cells associated with fluid space by contacting wound site situated in tissue associated with fluid space, with composition comprising nucleic acid and biocompatible matrix.
DC A96 B04 D16
IN PIERCE, G; SOSNOWSKI, B A
PA (SELE-N) SELECTIVE GENETICS INC
CYC 102

PI WO 2003029429 A2 20030410 (200340)* EN 95
RW: AT BE BG CH CY CZ DE DK EA EE ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SK SL SZ TR TZ UG ZM ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT RO RU SD SE SG SI SK SL TJ TM TN TR TT TZ UA UG US UZ VC VN YU ZA ZM ZW
US 2003148979 A1 20030807 (200358)
EP 1438413 A2 20040721 (200447) EN
R: AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI SK TR
AU 2002343475 A1 20030414 (200461)

ADT WO 2003029429 A2 WO 2002-US31546 20021002; US 2003148979 A1 Provisional US 2001-327513P 20011003, US 2002-264284 20021002; EP 1438413 A2 EP 2002-780419 20021002, WO 2002-US31546 20021002; AU 2002343475 A1 AU 2002-343475 20021002

FDT EP 1438413 A2 Based on WO 2003029429; AU 2002343475 A1 Based on WO 2003029429

PRAI US 2001-327513P 20011003; US 2002-264284 20021002

AB WO2003029429 A UPAB: 20030624

NOVELTY - Transferring (M1) a nucleic acid molecule into cells associated with a fluid space, involves contacting a wound site with a composition (I) comprising a nucleic acid molecule and a biocompatible matrix, the wound site being situated in a tissue associated with the fluid space.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for:

(1) stimulating (M2) gene expression in cartilage progenitor cells located within a cartilage progenitor tissue site of an animal, involves contacting the tissue site with a composition comprising a chondrogenic gene and a biocompatible matrix;

(2) stimulating (M3) cartilage repair or regeneration, by implanting at a cartilage defective site a matrix-gene composition comprising a chondrogenic gene and a biocompatible matrix;

(3) treating (M4) arthritis, by implanting at a cartilage defective site a matrix- gene composition comprising chondrogenic gene and a biocompatible matrix;

(4) treating (M5) ischemic heart disease by implanting a matrix-gene composition comprising an angiogenic gene and a biocompatible matrix into an ischemic region; and

(5) a composition comprising multiple genes associated with a multi-partitional biocompatible matrix.

ACTIVITY - Vulnerary; Antiarthritic; Antiinflammatory; Vasotropic.

MECHANISM OF ACTION - Gene therapy. Influence of collagen-immobilized fibroblast growth factor (FGF) genes on muscle wound repair was examined using the rodent hind limb model. At day 1¼ following delivery of DNA(FGF2) formulated in a blend of 1% collagen and 1% gelatin, trichrome stains revealed that these matrices were well infiltrated by both mononuclear cells and elongated fibroblastoid cells. Many of these cells were organized around simple single-walled vessel, and may represent vascular precursors giving rise to neovasculature. The presence of erythrocytes with vessel lumens confirmed that these vessels were perfused. By day 21 post-treatment, in addition to microvasculature, well-organized muscular arterioles were also present. Skeletal muscle bundles were scattered throughout the collagen-gelatin matrix, which appeared to be reduced in volume over that seen at day 14. neither the residual matrix nor the surrounding tissue showed any signs of edema. Very similar observations were seen following the delivery of collagen-gelatin-DNA(FGF6) to muscle wounds, including the development of both micro- and macrovasculature. Delivery of the control transgene luciferase induced a much different response. Even at day 21, considerable collagen-gelatin matrix remained, and although a mononuclear cell infiltrate was present, blood- perfused vessels perfused were rare. infiltrating cells were organized into discrete areas, however the majority of these structures were not true vasculature in that they were not lined by a continuous endothelium and were not perfused with blood. Finally, delivery of FGF2 protein was seen to induce a limited angiogenic response comprised of small capillaries. Arteriogenesis similar to that induced by FGF2 or FGF6 gene delivery was not observed.

USE - (M1) is useful for transferring a nucleic acid molecule into cells associated with a fluid space. (M2) is useful for stimulating gene expression in cartilage progenitor cells located within a cartilage progenitor tissue site of an animal, where expression of the gene in the cell stimulates the cells to promote cartilage tissue repair or regeneration. The cartilage progenitor tissue site of an animal is a site of cartilage injury (a partial-thickness injury or a full-thickness injury), or is a cartilage cavity site, or is the result of surgery or the removal of cartilage tumor. The chondrogenic gene is in the form of plasmid DNA, a DNA insert within the genome of a recombinant **adenovirus**, a DNA insert within the genome of a recombinant adeno-associated virus

(AAV) or a DNA insert within the genome of a recombinant retrovirus. The chondrogenic gene is parathyroid hormone (PTH) gene, bone morphogenic factor (BMP) gene, a cartilage-derived morphogenic protein (CDMP) gene, a growth factor gene, a growth factor receptor gene (e.g, IGF receptor gene or MBP receptor gene), where the growth factor gene is fibroblast growth factor (FGF) gene, insulin-like growth factor (IGF) gene, hepatocyte growth factor (HGF) gene, a gene in the transforming growth factor (TGF) family of genes, epidermal growth factor (EGF) gene, connective tissue growth factor (CTGF) gene, leukemia inhibitory factor (LIF) gene, parathyroid hormone-related peptide (PTHrP) gene, platelet-derived growth factor (PDGF) gene, skeletal growth factor (SGF) gene, BIP gene, MP52 gene, chondromodulin gene, preferably basic FGF gene, IGF-I or IGF-II gene, TGF alpha , TGF beta 1 or TGF beta 2, BMP2, BMP3, BMP4, BMP5, BMP6, BMP7, BMP8, BMP9, BMP10, BMP11, BMP12 or BMP13 gene. (M3) is useful for stimulating cartilage repair or regeneration. (M4) is useful for treating arthritis, where the chondrogenic gene that is implanted is an IL-4 gene, or a gene that encodes either a ribozyme that cleaves mRNAs for an inflammation mediator, or an antisense nucleic acid that binds to mRNA for an inflammation mediator such as IL-1, IL-6, IL-8, TNF- alpha , granulocyte-macrophage colony stimulating factor (GM-CSF), a soluble receptor that binds to a mediator of inflammation, or an antibody or its fragment that binds to a mediator of inflammation. (M5) is useful for treating ischemic heart disease, where the angiogenic gene that is implanted is FGF gene, VEGF gene, TNF- alpha gene, HGF gene, or a PDGF gene (all claimed).

ADVANTAGE - Direct plasmid DNA transfer from a matrix to a mammalian repair cell, through stimulation of the wound healing process, has the following advantages:

(a) each are capable of producing and purifying DNA constructs;

(b) matrices can act as structural scaffolds that, in and of themselves, promote cell in growth and proliferation, thus facilitating the targeting of repair cells for gene transfer;

(c) the introduction of a biocompatible matrix to tissues associated with a fluid space results in less damage to surrounding tissues during introduction;

(d) the biocompatible matrix may be implanted through or across the fluid space without harming other tissue;

(e) the method therefore, is a minimally invasive means of utilizing gene therapy to introduce therapeutic molecules to tissues associated with fluid spaces;

(f) the proximity of a fluid space facilitates the migration of repair cells to the biocompatible matrix that is inserted into a tissue associated with a fluid space; and

(g) the methods are efficient in introducing gene therapy products to target cells associated with a fluid space.

Dwg.0/6

L14 ANSWER 7 OF 14 WPIDS COPYRIGHT 2005 THE THOMSON CORP on STN
Full Text
AN 2002-582334 [62] WPIDS
DNC C2002-164580
TI Purifying **adenovirus** comprising a therapeutic gene from contaminants in a sample pool, by contacting sample pool with **hydroxyapatite** chromatographic medium to reversibly bind **adenovirus** and eluting the bound **adenovirus**.
DC B04 D16
IN CANNON-CARLSON, S V; CUTLER, C; VELLEKAMP, G J; VOLOCH, M; CUTLER, C M
PA (SCHE) SCHERING CORP
CYC 97
PI US 2002064860 A1 20020530 (200262)* 5
WO 2002044348 A2 20020606 (200262) EN
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZM ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CZ DE DK DM DZ EC EE ES FI GB GD GE HR HU ID IL IN IS JP KG KR KZ LC LK LR LT LU LV MA MD MG MK MN MX MZ NO NZ PH PL PT RO RU SE SG SI SK SL TJ TM TR TT TZ UA UZ VN YU ZA ZM
AU 2002032443 A 20020611 (200264)
EP 1337627 A2 20030827 (200357) EN
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI TR
ADT US 2002064860 A1 Provisional US 2000-253823P 20001129, US 2001-991080 20011116; WO 2002044348 A2 WO 2001-US44684 20011128; AU 2002032443 A AU 2002-32443 20011128; EP 1337627 A2 EP 2001-991967 20011128, WO 2001-US44684 20011128
FDT AU 2002032443 A Based on WO 2002044348; EP 1337627 A2 Based on WO 2002044348
PRAI US 2000-253823P 20001129; US 2001-991080 20011116
AB US2002064860 A UPAB: 20021031

NOVELTY - Purifying **adenovirus** from contaminants in a sample pool, comprises contacting the sample pool with a **hydroxyapatite** chromatographic medium to reversibly bind the **adenovirus** to the **hydroxyapatite** and eluting the bound **adenovirus** from the **hydroxyapatite**.

USE - The method is useful for purifying an **adenovirus** comprising a therapeutic gene or a nucleic acid sequence from the p53 or p21 gene, from contaminants in a sample pool. The **adenovirus** is preferably ACN53 (claimed). The **adenovirus** can be wild-type, recombinant, or mutant, type 2 and type 5 **adenoviral** vectors being preferred.

ADVANTAGE - The method reduces concentration of a contaminant in the sample pool by at least 80%, empty capsids by at least 75% or bovine serum albumin (BSA) by at least 70% (claimed). The method greatly reduces the level of contaminants and empty **adenovirus** capsids from **adenovirus** samples first purified by conventional purification methods. Empty capsids, i.e. incomplete viral particles which are not infectious and thus not useful for gene therapy or other applications, are separated from intact viruses. NaCl concentrations (150 mM) present in buffers prevent the **adenovirus** from irreversibly binding to the **hydroxyapatite**. Highly pure **adenovirus** preparation is obtained.

Dwg.0/0

L14 ANSWER 8 OF 14 WPIDS COPYRIGHT 2005 THE THOMSON CORP on STN
Full Text
AN 2002-171026 [22] WPIDS
CR 2000-423420 [36]; 2001-300278 [31]; 2002-689759 [74]; 2003-328485 [31]; 2003-370630 [35]; 2004-303322 [28]
DNC C2002-052770
TI Promoting growth of bone, ligament or cartilage in a mammal, involves administering to the mammal a protein which comprises growth factor domain of zvegf3 protein, a homolog of platelet-derived growth factor.
DC B04 D16
IN GILBERTSON, D G; HART, C E
PA (GILB-I) GILBERTSON D G; (HART-I) HART C E; (ZYMO) ZYMOGENETICS INC
CYC 1
PI US 2002004225 A1 20020110 (200222)* 31
US 6663870 B2 20031216 (200382)
ADT US 2002004225 A1 Provisional US 1998-111173P 19981207, Provisional US 1999-142576P 19990706, Provisional US 1999-161653P 19991021, Provisional US 1999-165255P 19991112, CIP of US 1999-457066 19991207, Provisional US 2000-193723P 20000331, US 2001-823033 20010329; US 6663870 B2 Provisional US 1998-111173P 19981207, Provisional US 1999-142576P 19990706, Provisional US 1999-161653P 19991021, Provisional US 1999-165255P 19991112, CIP of US 1999-457066 19991207, Provisional US 2000-193723P 20000331, US 2001-823033 20010329
PRAI US 2001-823033 20010329; US 1998-111173P 19981207;
US 1999-142576P 19990706; US 1999-161653P 19991021;
US 1999-165255P 19991112; US 1999-457066 19991207;
US 2000-193723P 20000331
AB US2002004225 A UPAB: 20040429
NOVELTY - Promoting (M) growth of bone, ligament or cartilage and stimulating proliferation of osteoblasts or chondrocytes in a mammal, comprises administering to the mammal a composition containing a dimeric protein (DP) which comprises growth factor domain comprising residues 235-345 of human or mouse zvegf3 protein of 345 (S1) amino acids fully defined in the specification, and a delivery vehicle.

DETAILED DESCRIPTION - Promoting growth of bone, ligament or cartilage in a mammal, involves administering to the mammal a protein which comprises growth factor domain of zvegf3 protein, which is a homolog of platelet-derived growth factor. Alternatively, in in vitro method, cartilage growth is promoted by culturing chondrocytes ex vivo in the presence of DP under conditions, where the chondrocytes proliferate, and placing the cultured chondrocytes into a mammal where cartilage is to be grown. Proliferation or differentiation of osteoblasts, osteoclasts, chondrocytes or bone marrow stem cells is promoted by culturing the cells in an effective amount of DP.

ACTIVITY - Osteopathic.

MECHANISM OF ACTION - Promoter of cell proliferation and differentiation. Recombinant zvegf3 was analyzed for mitogenic activity on human aortic smooth muscle cells (HAoSMC) and human umbilical vein endothelial cells (HUVEC). HAoSMC and HUVEC were plated at a density of 5000 cells/well in 96-well culture plates and grown for 24 hours in DMEM (Dulbecco's Modified Eagle medium) containing 10% fetal calf serum at 37 deg. C. Cells were quiesced by incubating them for 24 hours in serum-free DMEM/Ham's F-12 medium containing insulin (5 micro g/ml), transferrin (20 micro g/ml) and selenium (16 pg/ml) (ITS). Test samples consisted of either conditioned media (CM) from **adenovirally**-infected HaCaT human keratinocyte cells expressing full-length zvegf3, purified growth factor domain expressed in BHK cells, or control media from cells infected with parental **adenovirus**. The control CM was generated from HaCaT cells

infected with a parental green fluorescent protein-expressing **adenovirus** and treated identically to the zvegf3 CM. Purified protein in a buffer containing 0.1% bovine serum albumin (BSA) was serially diluted into ITS medium at concentrations of 1 micro g/ml-1 ng/ml and added to the test plate. A control buffer of 0.1% BSA was diluted identically to the highest concentration of zvegf3 protein and added to the plate. For measurement of (3H) thymidine incorporation, 20 micro l of a 50 micro Ci/ml stock in DMEM was added directly to the cells, for a final activity of 1 micro Ci/well. Mitogenic activity was assessed by measuring the uptake of (3H) thymidine. The results demonstrated that zvegf3 CM had approx. 1.5-fold higher mitogenic activity on HAoSM cells over control CM, and purified protein caused a maximal 1.8-fold increase in (3H) thymidine incorporation over the buffer control. zvegf3 CM had no mitogenic activity on HUVEC compared to the control CM, and purified protein caused a maximal 1.3-fold increase in (3H) thymidine incorporation over the buffer control.

USE - (M) is useful for promoting growth of bone, ligament or cartilage in a mammal, where the composition is administered at a site of a bony defect, preferably a fracture, bone graft site, implant site, or periodontal pocket, and for stimulating proliferation of osteoblasts or chondrocytes in a mammal. (M) is further useful for promoting proliferation of osteoblasts, osteoclasts, chondrocytes or bone marrow stem cells, where the bone marrow stem cells are harvested from a patient prior to culture (claimed). The method is therefore useful for treating osteoporosis.

Dwg.0/2

L14 ANSWER 9 OF 14 WPIDS COPYRIGHT 2005 THE THOMSON CORP on STN
Full Text
AN 2001-611088 [70] WPIDS
CR 2000-687541 [67]; 2002-573696 [61]; 2003-352153 [33]; 2003-874621 [81]; 2004-070738 [07]; 2004-225671 [21]; 2005-011673 [01]; 2005-161610 [17]
DNN N2005-258282 DNC C2005-098290
TI Use of zvegf4 polypeptide for promoting bone, ligament or cartilage growth in mammal at site of fracture, implant, and bone graft, and for promoting growth or differentiation of osteoblasts, chondrocytes in culture.
DC A96 B04 D16 P34
IN GILBERTSON, D G; HART, C E
PA (ZYMO) ZYMOGENETICS INC
CYC 92
PI WO 2001057083 A1 20010809 (200170)* EN 57
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ TZ UG ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG UZ VN YU ZA ZW
AU 2000051259 A 20010814 (200173)
US 6468543 B1 20021022 (200277)
ADT WO 2001057083 A1 WO 2000-US12095 20000503; AU 2000051259 A AU 2000-51259 20000503; US 6468543 B1 Provisional US 1999-132250P 19990503, Provisional US 1999-164463P 19991110, Provisional US 2000-180169P 20000204, US 2000-540224 20000331
FDT AU 2000051259 A Based on WO 2001057083
PRAI US 2000-540224 20000331; US 2000-180169P 20000204;
US 1999-132250P 19990503; US 1999-164463P 19991110
AB WO 200157083 A UPAB: 20050524
NOVELTY - Use of zvegf4 polypeptide (I) for promoting bone, ligament or cartilage growth in a mammal, and for promoting proliferation or differentiation of osteoblasts, osteoclasts, chondrocytes or bone marrow stem cells in culture. For promoting cartilage growth, chondrocytes are cultured ex vivo in presence of (I) and then placed into mammal where cartilage is to be grown.

DETAILED DESCRIPTION - Use of zvegf4 polypeptide (I):

(a) for promoting growth of bone, ligament or cartilage in a mammal when administered in combination with a delivery vehicle; and

(b) for promoting proliferation or differentiation of osteoblasts, osteoclasts, chondrocytes or bone marrow stem cells in culture. Promoting cartilage growth involves culturing chondrocytes ex vivo in the presence of (I) such that the chondrocytes proliferate and placing the cultured chondrocytes into a mammal where cartilage is to be grown.

ACTIVITY - Osteopathic; vulnerary.

MECHANISM OF ACTION - Production of bone and/or connective tissue stimulator; mitogenic; growth of bone, ligament or cartilage promoter. Recombinant zvegf4 was analyzed for mitogenic activity on rat liver stellate cells, human aortic smooth muscle cells, human retinal pericytes and human hepatic fibroblasts. Test samples consisted of conditioned media (CM) from **adenovirally** infected HaCaT human keratinocyte cells expressing full length zvegf-4. Control CM was generated from HaCaT cells infected with a parental green fluorescent protein (GFP)-expressing

adenovirus (zPar). The CM were concentrated 10-fold then diluted back to 1x with ITS media (serum-free DMEM/Ham's F-12 medium containing 5 micro g/ml insulin, 20 micro g/ml transferrin, and 16 pg/ml selenium). Cells were plated and grown for approximately 72 hours in DMEM (Dulbecco's modified eagles medium) containing 10% fetal calf serum at 37 deg. C. Cells were quiesced by incubating them for approximately 20 hours in serum-free DMEM/Ham's F-12 medium containing insulin, transferrin and selenium. At the time of the assay, the medium was removed, and test samples were added to the wells. After another 24 hours incubation, media were removed and cells were incubated with 0.1 ml of trypsin until cells detached. Cells were harvested. The plates were then dried, sealed after adding scintillation cocktail and counted on a microplate scintillation counter. Results demonstrated that zvegf4 CM had approximately 7-fold higher mitogenic activity than control CM on pericytes cells and approximately a 1.5-2.4-fold higher mitogenic activity on the other cell types tested.

USE - Zvegf4 polypeptide is useful for promoting growth of bone, ligament or cartilage in a mammal at a site of bony defect such as fracture, bone graft, implant or periodontal pocket (claimed), in humans and non-human animals such as domestic animals including livestock and companion animals. (I) is used for promoting growth of bone, ligament, or cartilage in conditions of bone defects following therapeutic treatments of bone cancers or other conditions characterized by increased bone loss or decreased bone formation, or elevation of peak bone mass in pre-menopausal woman. (I) is also useful for healing bone following radiation-induced osteonecrosis, repairing bone defects arising from surgery, and promotion of bone healing in plastic surgery, increasing bone formation during distraction osteogenesis, treating bone injuries including repair of cartilage and ligament and treatment of osteoporosis.
Dwg.0/1

L14 ANSWER 10 OF 14 WPIDS COPYRIGHT 2005 THE THOMSON CORP on STN
Full Text
AN 2001-407594 [43] WPIDS
DNC C2001-123425
TI Transferring a gene of interest to invertebral disc cells, is used to treat and study degenerative disc diseases.
DC A96 B04 D16
IN EVANS, C H; KANG, J D; NISHIDA, K; ROBBINS, P D
PA (EVAN-I) EVANS C H; (KANG-I) KANG J D; (NISH-I) NISHIDA K; (ROBB-I) ROBBINS P D
CYC 1
PI US 2001006948 A1 20010705 (200143)* 15
ADT US 2001006948 A1 US 1998-199978 19981125
PRAI US 1998-199978 19981125
AB US2001006948 A UPAB: 20010801
NOVELTY - Transferring a gene of interest to an invertebral disc cell, comprising inserting a nucleic acid encoding the gene into the cell so that it is expressed, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) treating a degenerative disc disease, comprising introducing a nucleic acid sequence encoding a gene of interest into a population of invertebral disc cells;

(2) producing an animal model for degenerative disc disease, comprising introducing a nucleic acid sequence encoding a gene of interest into a population of invertebral disc cells, so that expression of the gene in the animal contributes to a pathology of a degenerative disc disease; and

(3) a genetically modified invertebral disc cell which expresses an exogenous gene of interest.

ACTIVITY - Osteopathic.

MECHANISM OF ACTION - Gene therapy.

No biological data is given.

USE - For treating and studying degenerative disc disorders (claimed).
Dwg.0/4

L14 ANSWER 11 OF 14 WPIDS COPYRIGHT 2005 THE THOMSON CORP on STN
Full Text
AN 2001-389951 [41] WPIDS
DNC C2001-118827
TI Bioreactor for systemic delivery of bioactive agents, comprises nucleic acids encoding growth stimulating and bioactive agents, and a biocompatible substance capable of cellular infiltration.
DC A14 A17 A28 A89 B04 B07 D16 D22
IN CHANDLER, L A; PIERCE, G
PA (SELE-N) SELECTIVE GENETICS INC; (CHAN-I) CHANDLER L A; (PIER-I) PIERCE G
CYC 94

PI WO 2001040272 A2 20010607 (200141)* EN 69
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW
AU 2001019398 A 20010612 (200154)
US 2001044413 A1 20011122 (200176)
ADT WO 2001040272 A2 WO 2000-US32754 20001130; AU 2001019398 A AU 2001-19398 20001130; US 2001044413 A1 Provisional US 1999-168470P 19991201, US 2000-729644 20001130
FDT AU 2001019398 A Based on WO 2001040272
PRAI US 1999-168470P 19991201; US 2000-729644 20001130
AB WO 200140272 A UPAB: 20010724
NOVELTY - An in situ bioreactor (I) adapted for systemic delivery of bioactive agents, comprising a nucleic acid encoding a growth stimulating agent, a nucleic acid encoding a bioactive agent, and a biocompatible substance capable of cellular infiltration, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) systemic delivery of a protein from a tissue site in an animal, comprising contacting the tissue site with (I);

(2) a Bi-gene device comprising a biocompatible substance capable of cellular infiltration, a nucleic acid encoding a cell growth stimulating agent, and a second nucleic acid encoding a bioactive agent;

(3) a kit for the production of a device comprising:
(a) a container;
(b) a biocompatible substance;
(c) a nucleic acid encoding a cell growth stimulating agent; and
(d) a second nucleic acid encoding a bioactive agent; and
(4) a kit for the production of a coated device comprising:
(a) a device coated with a biocompatible substance;
(b) a nucleic acid encoding a growth stimulating agent; and
(c) a second nucleic acid encoding a bioactive agent.

ACTIVITY - Vulnerary; hemostatic; antianemic; antidiabetic; antiarthritic; coagulant; antiinflammatory; immunosuppressive; neuroprotective; cytostatic; antirheumatic; osteopathic; anti-infertility; contraception.

MECHANISM OF ACTION - Bioactive agent deliverer; protein and gene therapy.

USE - (I) is used for cellular ingrowth and systemic delivery of a bioactive agent, such as a protein from a tissue site in an animal (claimed). (I) is used as an implant. (I) can be used to treat conditions associated with renal dialysis, hemophilia, hemoglobinopathies, thalassemias, anemia, lipid storage disease, mucopolysaccharidoses, diabetes, hypercoagulability, arthritis, hypercoagulability, stroke, cerebroprotective, inflammation, infection, autoimmunity, multiple sclerosis, thrombocytopenia, cancer, osteoporosis, infertility, and birth control.

ADVANTAGE - (I) allows sustained and controlled gene delivery as well as sustained product expression using in vivo transfer and expression of desired nucleic acids.

Dwg.0/3

L14 ANSWER 12 OF 14 WPIDS COPYRIGHT 2005 THE THOMSON CORP on STN
Full Text
AN 2001-202687 [20] WPIDS
DNN N2001-144640 DNC C2001-060149
TI Delivery of agents into targeted tissue, particularly cardiac tissue comprises a flowable substance containing a number of small particles.
DC A96 B05 B07 P31
IN EVANS, D G; HOGANSON, D M; NASH, J E
PA (KENS-N) KENSEY NASH CORP; (MARS-N) MARSH CREEK CORP CENT
CYC 95
PI WO 2001010313 A1 20010215 (200120)* EN
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TZ UG ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW
AU 2000066112 A 20010305 (200130)
EP 1206219 A1 20020522 (200241) EN
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI
JP 2003506131 W 20030218 (200315) 99
US 2003191449 A1 20031009 (200367)
US 6709427 B1 20040323 (200421)

US 2004158227 A1 20040812 (200454)
ADT WO 2001010313 A1 WO 2000-US20525 20000728; AU 2000066112 A AU 2000-66112 20000728; EP 1206219 A1 EP 2000-953711 20000728, WO 2000-US20525 20000728; JP 2003506131 W WO 2000-US20525 20000728, JP 2001-514842 20000728; US 2003191449 A1 Div ex US 1999-368410 19990805, US 2003-405394 20030402; US 6709427 B1 US 1999-368410 19990805; US 2004158227 A1 Cont of US 1999-368410 19990805, US 2004-763558 20040123
FDT AU 2000066112 A Based on WO 2001010313; EP 1206219 A1 Based on WO 2001010313; JP 2003506131 W Based on WO 2001010313; US 2004158227 A1 Cont of US 6709427
PRAI US 1999-368410 19990805; US 2003-405394 20030402; US 2004-763558 20040123
AB WO 200110313 A UPAB: 20010410
NOVELTY - A system (I) for delivering agents into a targeted internal tissue comprising a delivery instrument (II) and a flowable agent (III) containing a number of small particles for introduction into the tissue, where (II) is arranged to introduce (III) at or adjacent the tissue by imparting a force to (III) which enters the tissue at an entry sinus, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for (I) for vascularizing cardiac tissue to cause the formation of lumens in communication with the patient's arterial system and treating cardiac tissue to affect the conduction of electrical signals or nerve signals in the cardiac tissue.

USE - For delivering agents into targeted tissue, particularly cardiac tissue (claimed).

ADVANTAGE - The local intra-tissue delivery of the flowable agent is more efficient than previous systems for delivery of medications to the heart, i.e. systemically by vein or regionally, e.g. intracoronary infusion.

Dwg.0/22

L14 ANSWER 13 OF 14 WPIDS COPYRIGHT 2005 THE THOMSON CORP on STN
Full Text
AN 2000-524218 [47] WPIDS
DNN N2000-387494 DNC C2000-155662
TI Composition for delivery of a virus vector to an animal cell comprising a virus vector bound to the exterior surface of a matrix, useful for gene therapy of conditions such as cancer and wounds.
DC A96 B04 B07 D16 D22 P31
IN JONES, P L; LEVY, R J
PA (CHIL-N) CHILDRENS HOSPITAL PHILADELPHIA
CYC 90
PI WO 2000043044 A1 20000727 (200047)* EN 83
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ TZ UG ZW
W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW
AU 2000034714 A 20000807 (200055)
ADT WO 2000043044 A1 WO 2000-US1193 20000119; AU 2000034714 A AU 2000-34714 20000119
FDT AU 2000034714 A Based on WO 2000043044
PRAI US 1999-116405P 19990119
AB WO 200043044 A UPAB: 20000925
NOVELTY - A composition for delivery of a virus vector to an animal cell comprising a virus vector bound to the exterior surface of a matrix in a physiologically reversible manner, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a surface coated with the composition above;

(2) an implantable device having a surface coated with the composition;

(3) a method (M1) of making a composition for delivery of a virus vector to an animal; and

(4) a method (M2) of delivering a virus vector to an animal tissue, comprising placing the composition in fluid communication with the animal tissue.

ACTIVITY - Vulnerary; cytostatic; vasotropic.

MECHANISM OF ACTION - Gene therapy.

USE - The composition is used to deliver nucleic acids encoding anti-restenotic and, anti-oncogenic proteins into cells, particularly in gene therapy of disorders such as wounds, cancer and restenosis.

Dwg.0/1

L14 ANSWER 14 OF 14 WPIDS COPYRIGHT 2005 THE THOMSON CORP on STN
Full Text
AN 2000-412321 [35] WPIDS

CR 2005-081936 [09]
DNN N2000-308172 DNC C2000-125048
TI Nucleic acids (I) encoding a transforming growth factor beta binding protein, useful for identifying agents for treating osteopenia, osteoporosis and fractures.
DC B04 D16 P14 S03
IN BRUNKOW, ME; GALAS, D J; KOVACEVICH, B; MULLIGAN, J T; PAEPER, B W; VAN NESS, J; WINKLER, D G; BRUNKOW, M E; PAEPER, B; NESS, J V
PA (DARW-N) DARWIN DISCOVERY LTD; (CLLT) CELLTECH R & D INC
CYC 91
PI WO 2000032773 A1 20000608 (200035)* EN 157
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ TZ UG ZW
W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW
AU 2000020313 A 20000619 (200044)
EP 1133558 A1 20010919 (200155) EN
R: AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE
BR 9915679 A 20020305 (200225)
CN 1333828 A 20020130 (200231)
US 6395511 B1 20020528 (200243)
JP 2002531090 W 20020924 (200278) 195
US 6489445 B1 20021203 (200301)
US 6495736 B1 20021217 (200307)
ZA 2001004234 A 20030226 (200321) 189
US 2003166247 A1 20030904 (200359)
NZ 512122 A 20031219 (200404)
US 2004009535 A1 20040115 (200406)
MX 2001005275 A1 20030601 (200417)
US 2004058321 A1 20040325 (200422)
AU 2003271274 A1 20040122 (200442)#
AU 769977 B2 20040212 (200453)
US 2004158045 A1 20040812 (200454)
US 6803453 B1 20041012 (200467)
ADT WO 2000032773 A1 WO 1999-US27990 19991124; AU 2000020313 A AU 2000-20313 19991124; EP 1133558 A1 EP 1999-963986 19991124, WO 1999-US27990 19991124; BR 9915679 A BR 1999-15679 19991124, WO 1999-US27990 19991124; CN 1333828 A CN 1999-815505 19991124; US 6395511 B1 Provisional US 1998-110283P 19981127, US 1999-449218 19991124; JP 2002531090 W WO 1999-US27990 19991124, JP 2000-585404 19991124; US 6489445 B1 Provisional US 1998-110283P 19981127, Div ex US 1999-449218 19991124, US 2000-668529 20000921; US 6495736 B1 Provisional US 1998-110283P 19981127, Div ex US 1999-449218 19991124, US 2000-668037 20000921; ZA 2001004234 A ZA 2001-4234 20010523; US 2003166247 A1 Provisional US 1998-110283P 19981127, Cont of US 1999-449218 19991124, Div ex US 2002-95248 20020307, US 2003-384893 20030306; NZ 512122 A NZ 1999-512122 19991124, WO 1999-US27990 19991124; US 2004009535 A1 Provisional US 1998-110283P 19981127, Cont of US 1999-449218 19991124, CIP of US 2002-95248 20020307, US 2003-463190 20030616; MX 2001005275 A1 WO 1999-US27990 19991124, MX 2001-5275 20010525; US 2004058321 A1 Provisional US 1998-110283P 19981127, Cont of US 1999-449218 19991124, US 2002-95248 20020307; AU 2003271274 A1 Div ex AU 2000-20313 19991124, AU 2003-271274 20031218; AU 769977 B2 AU 2000-20313 19991124; US 2004158045 A1 Provisional US 1998-110283P 19981127, Div ex US 1999-449218 19991124, Cont of US 2000-668021 20000921, US 2004-788606 20040227; US 6803453 B1 Provisional US 1998-110283P 19981127, Div ex US 1999-449218 19991124, US 2000-668021 20000921
FDT AU 2000020313 A Based on WO 2000032773; EP 1133558 A1 Based on WO 2000032773; BR 9915679 A Based on WO 2000032773; JP 2002531090 W Based on WO 2000032773; US 6489445 B1 Div ex US 6395511; US 6495736 B1 Div ex US 6395511; US 2003166247 A1 Cont of US 6395511; NZ 512122 A Div in NZ 529538, Based on WO 2000032773; US 2004009535 A1 Cont of US 6395511; MX 2001005275 A1 Based on WO 2000032773; US 2004058321 A1 Cont of US 6395511; AU 769977 B2 Previous Publ. AU 2000020313, Based on WO 2000032773; US 2004158045 A1 Div ex US 6395511; US 6803453 B1 Div ex US 6395511
PRAI US 1998-110283P 19981127; US 1999-449218 19991124;
US 2000-668529 20000921; US 2000-668037 20000921;
US 2002-95248 20020307; US 2003-384893 20030306;
US 2003-463190 20030616; AU 2003-271274 20031218;
US 2000-668021 20000921; US 2004-788606 20040227
AB WO 200032773 A UPAB: 20050207
NOVELTY - Isolated nucleic acids (I) encoding a transforming growth factor beta binding protein (TGF- beta BP) (II), are new. claims are also included for a ribozyme capable of cleaving TGF- beta BP mRNA.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following:
(1) an isolated nucleic acid molecule (I) selected from:
(a) an isolated nucleic acid molecule comprising 1 of 6 defined

sequences ((Ia) - (Ie)) given in the specification (or their complements);

(b) an isolated nucleic acid molecule that specifically hybridizes to the nucleic acid molecule of (a) under conditions of high stringency; and

(c) an isolated nucleic acid molecule that encodes a TGF- beta binding protein (BP) according to (a) or (b);

(2) an isolated oligonucleotide (I') which hybridizes to (Ia) - (Ig) ((If) and (Ig) are defined sequences given in the specification), or their complements under high stringency conditions;

(3) an isolated protein (II) comprising a TGF- beta BP encoded by (I);

(4) an expression vector (III) comprising a promoter operably linked to (I);

(5) a method (M1) of producing a TGF- beta BP (II), comprising culturing a cell (IV) which contains (III) under conditions suitable for protein production;

(6) a host cell (IV) comprising (III);

(7) an antibody (V) that specifically binds to (II);

(8) a hybridoma (VI) that produces (V);

(9) a fusion protein (VII) comprising (II) (or a fragment of (II) at least 10 amino acids in length) and a second polypeptide comprising a non-TGF- beta BP.

(10) a pair of primers (VIII) that specifically amplify all or part of (I);

(11) a ribozyme (IX) which cleaves RNA encoding (II);

(12) a nucleic acid molecule (X) encoding (IX);

(13) a vector (XI) comprising (X);

(14) a host cell (XII) comprising (X) and/or (XI);

(15) a method (M2) for producing a ribozyme, comprising providing (X) under the transcriptional control of a promoter and transcribing the DNA to produce (IX);

(16) a method (M3) for increasing bone mineralization, comprising introducing (IX) into a warm blooded animal;

(17) a method (M4) of detecting nucleic acids encoding TGF- beta BP, comprising incubating (I') under high stringency conditions, and detecting hybridization of the oligonucleotide;

(18) a method (M5) for detecting a TGF- beta , comprising incubating (V) under conditions suitable for binding to (II) and detecting any binding that occurs;

(19) a transgenic non-human animal (XIII) whose germ cells and somatic cells contain a nucleic acid molecule encoding a TGF- beta BP operably linked to a promoter (the gene is introduced into the animal (or an ancestor) at an embryonic stage);

(20) a method (M6) for determining whether a candidate molecule is capable of increasing bone mineral content, comprising:

(a) mixing 1 or more candidate molecules (CMs) with TGF- beta BP encoded by (I) and a selected member of the TGF- beta protein family; and

(b) determining whether the CM alters the signaling of the TGF- beta protein or alters the binding of the TGF- beta BP to the TGF- beta protein;

(21) a kit (XIV) for the detection of TGF- beta BP expression comprising (I), (I') and/or a 20 nucleotide fragment of (I); and

(22) a kit (XV) for the detection of TGF- beta BP comprising (V).

ACTIVITY - Osteopathic.

MECHANISM OF ACTION - Transforming growth factor- beta binding protein.

The interaction of BEER (a TGF- beta BP) with proteins from different phylogenetic arms of the TGF- beta superfamily were studied using immunoprecipitation methods. Purified TGF- beta 1, TGF- beta 2, TGF- beta 3, BMP-4, BMP-5, BMP-6 and GDNF were obtained from commercial sources. Partially purified BEER was dialyzed into HEPES (N-(2-OH-ethyl-)piperazine-N'-(2-ethanesulfonic acid)) buffered saline (25 mM HEPES 7.5, 150 mM NaCl). Immunoprecipitations were done in 300 microliters of IP buffer (150 mM NaCI, 25 mM Tris pH 7.5, 1 mM EDTA (ethylene diamine tetraacetic acid), 1.4 mM beta -mercaptoethanol, 0.5 % triton multiply 100, and 10% glycerol). 30 ng recombinant human BMP-5 protein was applied to 15 microliters of FLAG affinity matrix in the presence and absence of 500 ng FLAG epitope-tagged BEER. The proteins were incubated for 4 hours at 4 deg. C and then the affinity matrix-associated proteins were washed 5 times in IP buffer (1 ml per wash). The bound proteins were eluted from the affinity matrix in 60 microliters of 1 multiply SDS PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) sample buffer. The proteins were resolved by SDS PAGE and BEER associated BNP-5 was detected by western blot using anti-BNP-5 antiserum.

USE - (I) and the protein (II) it encodes may be used in the prevention, treatment and diagnosis of diseases associated with inappropriate TGF-beta BP expression. For example, (I) (and vectors containing (I) (III)) and the TGF-beta BP polypeptide may be used to treat disorders associated with decreased TGF-beta BP expression.

(I) or (III) may be administered to treat diseases by rectifying

mutations or deletions in a patient's genome that affect the activity of TGF-beta BP by expressing inactive proteins or to supplement the patients own production of TGF-beta BP polypeptides. Additionally, (I) may be used to produce TGF-beta BP, according to standard recombinant DNA methodology (for example see Sambrook et al., Molecular Biology: A Laboratory Manual, (1989)), by inserting the nucleic acids into a host cell and culturing the cell to express the protein (the protein may be expressed either in vitro (in a fermentation culture) or in vivo (as part of a gene therapy procedure)). Conversely, antisense nucleic acid molecules (i.e. (I')) may be administered to down regulate TGF-beta BP expression by binding with the cells own TGF-beta BP genes and preventing their expression.

(I) and (I') may also be used as DNA probes in diagnostic assays (e.g. polymerase chain reactions (PCR)) to detect and quantitate the presence of similar nucleic acid sequences in samples, and hence which patients may be in need of restorative therapy.

They may also be used to study the expression and function of TGF-beta BP polypeptides and their role in metabolism.

The TGF-beta BP polypeptides may be used as antigens in the production of antibodies against TGF-beta BP and in assays to identify modulators (agonists and antagonists) of TGF-beta BP expression and activity. The anti-TGF-beta BP antibodies and TGF-beta BP antagonists may also be used to down regulate TGF-beta BP expression and activity.

The anti-TGF-beta BP antibodies may also be used as diagnostic agents for detecting the presence of TGF-beta BP polypeptides in samples (e.g. by enzyme linked immunosorbant assay (ELISA)). Antagonists of TGF-beta BP may be used to increase bone mineral content for the treatment of conditions such a osteopenia, osteoporosis, fractures and other disorders associated with low mineral content.

The ribozyme may be used for cleaving TGF-beta BP mRNA.

Dwg.0/6

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=> e shabram p w/in
E1          5     SHABRAM P/IN
E2          1     SHABRAM P L/IN
E3          6 --> SHABRAM P W/IN
E4          7     SHABRANG M/IN
E5          3     SHABRANSKI V A/IN
E6          1     SHABRAT Y U A/IN
E7          6     SHABRATOV D V/IN
E8          1     SHABRATOV V G/IN
E9          1     SHABRATSK V I/IN
E10         5     SHABRATSKI V I/IN
E11         4     SHABRATSKII V I/IN
E12         1     SHABRATSKIVI/IN

=> s e3
L15         6 "SHABRAM P W"/IN

=> d l15,bib,1-6

L15  ANSWER 1 OF 6  WPIDS  COPYRIGHT 2005 THE THOMSON CORP on STN
Full Text
AN   2003-198263 [19]   WPIDS
DNC  C2003-050756
TI   New recombinant virus utilizing a pathway-responsive promoter that
     inhibits viral replication, useful for diagnosing and treating cancer with
     p53-pathway defects or with a lack of TGFapproximatelyb antiproliferative
     action.
DC   B04 D16
IN   RAMACHANDRA, M; SHABRAM, P W
PA   (RAMA-I) RAMACHANDRA M; (SHAB-I) SHABRAM P W
CYC  1
PI   US 2002150557   A1 20021017 (200319)*      51
ADT  US 2002150557 A1 Provisional US 1998-104399P 19981015, CIP of US
     1999-416812 19991013, US 2002-62216 20020130
PRAI US 1998-104399P      19981015; US 1999-416812       19991013;
     US 2002-62216        20020130

L15  ANSWER 2 OF 6  WPIDS  COPYRIGHT 2005 THE THOMSON CORP on STN
Full Text
AN   2001-191764 [19]   WPIDS
DNC  C2001-057587
TI   Reducing antiviral antibodies concentration in a plasma sample with a
     immunoaffinity chromatographic column to allow improved treatment of
     mammals with therapeutic viruses.
DC   B04 D16
IN   LAFACE, D M; RAHMAN, A; SHABRAM, P W; TSAI, V T
PA   (CANJ-N) CANJI INC; (LAFA-I) LAFACE D M; (RAHM-I) RAHMAN A; (SHAB-I)
```

SHABRAM P W; (TSAI-I) TSAI V T
CYC 93
PI WO 2001017537 A2 20010315 (200119)* EN 47
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ
NL OA PT SD SE SL SZ TZ UG ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CR CZ DE DK DM DZ
EE ES FI GB GD GE HR HU ID IL IN IS JP KG KR KZ LC LK LR LT LU LV
MA MD MG MK MN MX MZ NO NZ PL PT RO RU SE SG SI SK SL TJ TM TR TT
TZ UA UZ VN YU ZA
AU 2000074741 A 20010410 (200137)
EP 1214082 A2 20020619 (200240) EN
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT
RO SE SI
US 6464976 B1 20021015 (200271)
US 2002187143 A1 20021212 (200301)
JP 2003508157 W 20030304 (200319) 56
MX 2002002453 A1 20020801 (200367)
ADT WO 2001017537 A2 WO 2000-US24109 20000901; AU 2000074741 A AU 2000-74741 20000901; EP 1214082 A2 EP 2000-963306 20000901, WO 2000-US24109 20000901; US 6464976 B1 Provisional US 1999-152650P 19990907, US 2000-653474 20000831; US 2002187143 A1 Provisional US 1999-152650P 19990907, Div ex US 2000-653474 20000831, US 2002-222722 20020816; JP 2003508157 W WO 2000-US24109 20000901, JP 2001-521328 20000901; MX 2002002453 A1 WO 2000-US24109 20000901, MX 2002-2453 20020306
FDT AU 2000074741 A Based on WO 2001017537; EP 1214082 A2 Based on WO 2001017537; US 2002187143 A1 Div ex US 6464976; JP 2003508157 W Based on WO 2001017537; MX 2002002453 A1 Based on WO 2001017537
PRAI US 1999-152650P 19990907; US 2000-653474 20000831;
US 2002-222722 20020816

L15 ANSWER 3 OF 6 WPIDS COPYRIGHT 2005 THE THOMSON CORP on STN
Full Text
AN 2000-317990 [27] WPIDS
DNC C2000-096341
TI Recombinant viruses which selectively replicate in target cells used in the treatment of tumors comprise a pathway-responsive promoter linked to a viral replication repressor.
DC B04 C06 D16
IN RAMACHANDRA, M; **SHABRAM, P W**
PA (CANJ-N) CANJI INC
CYC 88
PI WO 2000022137 A2 20000420 (200027)* EN 49
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL
OA PT SD SE SL SZ TZ UG ZW
W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CZ DE DK DM EE ES FI
GB GD GE HR HU ID IL IN IS JP KG KR KZ LC LK LR LT LU LV MA MD MG
MK MN MX NO NZ PL PT RO RU SE SG SI SK SL TJ TM TR TT TZ UA UZ VN
YU ZA
AU 9963915 A 20000501 (200036)
BR 9914527 A 20010626 (200140)
NO 2001001843 A 20010614 (200141)
EP 1121441 A2 20010808 (200146) EN
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT
RO SE SI
SK 2001000441 A3 20011203 (200203)
CZ 2001001129 A3 20020116 (200215)
CN 1330715 A 20020109 (200229)
HU 2001004107 A2 20020328 (200234)
KR 2002013472 A 20020220 (200257)
JP 2002541761 W 20021210 (200301) 72
AU 758354 B 20030320 (200329)
MX 2001003840 A1 20020301 (200362)
NZ 510805 A 20031219 (200404)
AU 2003204841 A1 20030724 (200464)#
NZ 528283 A 20050527 (200537)
ADT WO 2000022137 A2 WO 1999-US21452 19991014; AU 9963915 A AU 1999-63915 19991014; BR 9914527 A BR 1999-14527 19991014, WO 1999-US21452 19991014; NO 2001001843 A WO 1999-US21452 19991014, NO 2001-1843 20010410; EP 1121441 A2 EP 1999-951481 19991014, WO 1999-US21452 19991014; SK 2001000441 A3 WO 1999-US21452 19991014, SK 2001-441 19991014; CZ 2001001129 A3 WO 1999-US21452 19991014, CZ 2001-1129 19991014; CN 1330715 A CN 1999-814361 19991014; HU 2001004107 A2 WO 1999-US21452 19991014, HU 2001-4107 19991014; KR 2002013472 A KR 2001-704690 20010413; JP 2002541761 W WO 1999-US21452 19991014, JP 2000-576027 19991014; AU 758354 B AU 1999-63915 19991014; MX 2001003840 A1 WO 1999-US21452 19991014, MX 2001-3840 20010416; NZ 510805 A NZ 1999-510805 19991014, WO 1999-US21452 19991014; AU 2003204841 A1 Div ex AU 1999-63915 19991014, AU 2003-204841 20030620; NZ 528283 A Div ex NZ 1999-510805 19991014, NZ 1999-528283 19991014

FDT AU 9963915 A Based on WO 2000022137; BR 9914527 A Based on WO 2000022137; EP 1121441 A2 Based on WO 2000022137; SK 2001000441 A3 Based on WO 2000022137; CZ 2001001129 A3 Based on WO 2000022137; HU 2001004107 A2 Based on WO 2000022137; JP 2002541761 W Based on WO 2000022137; AU 758354 B Previous Publ. AU 9963915, Based on WO 2000022137; MX 2001003840 A1 Based on WO 2000022137; NZ 510805 A Div in NZ 528283, Based on WO 2000022137; NZ 528283 A Div ex NZ 510805
PRAI US 1998-172686 19981015; AU 2003-204841 20030620

L15 ANSWER 4 OF 6 WPIDS COPYRIGHT 2005 THE THOMSON CORP on STN
Full Text
AN 2000-317859 [27] WPIDS
DNC C2000-096225
TI Use of calpain inhibitors to enhance p53-mediated apoptosis in a cell in combination with p53, to induce cell death in p53 positive tumor cells, and for ablating neoplastic cells contaminating a population of normal cells.
DC B04 D16
IN ATENCIO, I A; LAFACE, D M; RAMACHANDRA, M; **SHABRAM, P W**
PA (CANJ-N) CANJI INC
CYC 87
PI WO 2000021575 A2 20000420 (200027)* EN 53
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ TZ UG ZW
W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CZ DE DK DM EE ES FI GB GD GE HR HU ID IL IN IS JP KG KR KZ LC LK LR LT LU LV MA MD MG MK MN MX NO NZ PL PT RO RU SE SG SI SK SL TJ TM TR TT TZ UA UZ VN YU ZA
AU 2000010926 A 20000501 (200036)
ADT WO 2000021575 A2 WO 1999-US21453 19991014; AU 2000010926 A AU 2000-10926 19991014
FDT AU 2000010926 A Based on WO 2000021575
PRAI US 1998-172685 19981015

L15 ANSWER 5 OF 6 WPIDS COPYRIGHT 2005 THE THOMSON CORP on STN
Full Text
AN 2000-023581 [02] WPIDS
DNC C2000-005872
TI Achieving cell density in microcarrier based bioreactor process, useful for production of virus, especially for gene therapy.
DC B04 D16
IN GIROUX, D D; GOUDREAU, A M; RAMACHANDRA, M; **SHABRAM, P W**
PA (CANJ-N) CANJI INC
CYC 85
PI WO 9957297 A1 19991111 (200002)* EN 32
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ UG ZW
W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CZ DE DK EE ES FI GB GD GE HR HU ID IL IN IS JP KG KR KZ LC LK LR LT LU LV MD MG MK MN MX NO NZ PL PT RO RU SE SG SI SK SL TJ TM TR TT UA UZ VN YU ZA
US 5994134 A 19991130 (200003)
AU 9938823 A 19991123 (200016)
EP 1078095 A1 20010228 (200113) EN
R: AT BE CH DE DK ES FI FR GB GR IE IT LI LT LU LV NL PT RO SE
JP 2002513583 W 20020514 (200236) 41
MX 2000010892 A1 20020401 (200363)
ADT WO 9957297 A1 WO 1999-US9813 19990504; US 5994134 A US 1998-73076 19980504; AU 9938823 A AU 1999-38823 19990504; EP 1078095 A1 EP 1999-921681 19990504, WO 1999-US9813 19990504; JP 2002513583 W WO 1999-US9813 19990504, JP 2000-547250 19990504; MX 2000010892 A1 WO 1999-US9813 19990504, MX 2000-10892 20001106
FDT AU 9938823 A Based on WO 9957297; EP 1078095 A1 Based on WO 9957297; JP 2002513583 W Based on WO 9957297; MX 2000010892 A1 Based on WO 9957297
PRAI US 1998-73076 19980504

L15 ANSWER 6 OF 6 WPIDS COPYRIGHT 2005 THE THOMSON CORP on STN
Full Text
AN 1996-425436 [42] WPIDS
DNC C1996-134114
TI Purificn. of recombinant viral vectors contg. therapeutic genes from cell lysates - by, e.g. treating lysate with enzyme, chromatographing lysate with anion exchange resin and chromatographing this prod on immobilised metal ion resin.
DC A96 B04 D16
IN HUYGHE, B G; LIU, X; **SHABRAM, P W**; SHEPARD, H M
PA (CANJ-N) CANJI INC
CYC 72
PI WO 9627677 A2 19960912 (199642)* EN 41
RW: AT BE CH DE DK EA ES FI FR GB GR IE IT KE LS LU MC MW NL OA PT SD

SE SZ UG
W: AL AM AT AU AZ BB BG BR BY CA CH CN CZ DE DK EE ES FI GB GE HU IS
JP KE KG KP KR KZ LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT
RO RU SD SE SG SI SK TJ TM TR TT UA UG UZ VN
AU 9654213 A 19960923 (199702)
WO 9627677 A3 19970116 (199715)
ZA 9601849 A 19971126 (199802) 42
EP 813606 A1 19971229 (199805) EN
R: AT BE CH DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE
US 5837520 A 19981117 (199902)
JP 2000510682 W 20000822 (200045) 45
ADT WO 9627677 A2 WO 1996-US3369 19960306; AU 9654213 A AU 1996-54213 19960306; WO 9627677 A3 WO 1996-US3369 19960306; ZA 9601849 A ZA 1996-1849 19960306; EP 813606 A1 EP 1996-911282 19960306, WO 1996-US3369 19960306; US 5837520 A US 1995-400793 19950307; JP 2000510682 W JP 1996-527081 19960306, WO 1996-US3369 19960306
FDT AU 9654213 A Based on WO 9627677; EP 813606 A1 Based on WO 9627677; JP 2000510682 W Based on WO 9627677
PRAI US 1995-400793 19950307

=> d l15,bib,ab,6

L15 ANSWER 6 OF 6 WPIDS COPYRIGHT 2005 THE THOMSON CORP on STN
Full Text
AN 1996-425436 [42] WPIDS
DNC C1996-134114
TI Purificn. of recombinant viral vectors contg. therapeutic genes from cell lysates - by, e.g. treating lysate with enzyme, chromatographing lysate with anion exchange resin and chromatographing this prod on immobilised metal ion resin.
DC A96 B04 D16
IN HUYGHE, B G; LIU, X; **SHABRAM, P W**; SHEPARD, H M
PA (CANJ-N) CANJI INC
CYC 72
PI WO 9627677 A2 19960912 (199642)* EN 41
RW: AT BE CH DE DK EA ES FI FR GB GR IE IT KE LS LU MC MW NL OA PT SD SE SZ UG
W: AL AM AT AU AZ BB BG BR BY CA CH CN CZ DE DK EE ES FI GB GE HU IS JP KE KG KP KR KZ LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK TJ TM TR TT UA UG UZ VN
AU 9654213 A 19960923 (199702)
WO 9627677 A3 19970116 (199715)
ZA 9601849 A 19971126 (199802) 42
EP 813606 A1 19971229 (199805) EN
R: AT BE CH DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE
US 5837520 A 19981117 (199902)
JP 2000510682 W 20000822 (200045) 45
ADT WO 9627677 A2 WO 1996-US3369 19960306; AU 9654213 A AU 1996-54213 19960306; WO 9627677 A3 WO 1996-US3369 19960306; ZA 9601849 A ZA 1996-1849 19960306; EP 813606 A1 EP 1996-911282 19960306, WO 1996-US3369 19960306; US 5837520 A US 1995-400793 19950307; JP 2000510682 W JP 1996-527081 19960306, WO 1996-US3369 19960306
FDT AU 9654213 A Based on WO 9627677; EP 813606 A1 Based on WO 9627677; JP 2000510682 W Based on WO 9627677
PRAI US 1995-400793 19950307
AB WO 9627677 A UPAB: 19961115
Purificn. of a recombinant viral vector contg. a therapeutic gene from a cell lysate, comprises:

(a) treating the cell lysate with an enzymatic agent that selectively degrades both encapsulated DNA and RNA;

(b) chromatographing the treated lysate from step (a) on a first resin, and

(c) chromatographing the eluant from step (b) on a second resin.

One of the resins is an anion exchange resin and the other is an immobilised metal ion affinity resin.

Also claimed are:

(1) determining the number of intact viral particles in an eluant sample from a chromatographic procedure comprising:

(a') constructing a standard curve on the number of viral particles at a selected absorbance;

(b') chromatographing the sample contg. the viral particles on an anion exchange resin;

(c') monitoring the chromatography of step (b') at the selected absorbance, and

(d') determining the total number of viral particles in the eluant sample by comparing the absorbance value obtd. in step (c') to the standard curve of step (a').

USE - The viral vectors may be used in gene therapy.

ADVANTAGE - The processes (A) and (B) yield vectors which retain their infectivity during and after chromatographic treatment and are able to effect gene transfer. The purificn. processes yield vectors which are as pure and active as vectors purified using a three day ultracentrifugation.
Dwg.0/8

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=> d his

     (FILE 'HOME' ENTERED AT 18:36:36 ON 22 JUN 2005)

     FILE 'USPATFULL' ENTERED AT 18:36:44 ON 22 JUN 2005
L1          9532 S HYDROXYAPATITE
L2          1599 S L1 AND HYDROXYAPATITE/CLM
L3            69 S L2 AND ADENOVIR?
L4            13 S L3 AND ADENOVIR?/CLM
L5            33 S L3 AND AY<2001
L6            27 S L5 NOT L4
L7             1 S US5496926/PN
L8             1 S L7 AND ADENOVIR?
L9             1 S US5624833/PN
L10            1 S L9 AND ADENOVIR?
               E ERICKSON AMY E/IN
L11            1 S E3
               E SHABRAM PAUL W/IN
L12            5 S E3

     FILE 'WPIDS' ENTERED AT 18:46:16 ON 22 JUN 2005
L13         2918 S HYDROXYAPATITE
L14           14 S L13 AND ADENOVIR?
               E SHABRAM P W/IN
L15            6 S E3

=> e erickson a e/in
E1             4     ERICKSON A A/IN
E2             1     ERICKSON A B/IN
E3             1 --> ERICKSON A E/IN
E4             1     ERICKSON A G/IN
E5             2     ERICKSON A H/IN
E6             1     ERICKSON A I/IN
E7             4     ERICKSON A J/IN
E8             1     ERICKSON A K/IN
E9             3     ERICKSON A L/IN
E10            4     ERICKSON A N/IN
E11            2     ERICKSON A O/IN
E12           10     ERICKSON A R/IN

=> s e3
L16            1 "ERICKSON A E"/IN

=> d l16,bib,ab

L16  ANSWER 1 OF 1  WPIDS  COPYRIGHT 2005 THE THOMSON CORP on STN
Full Text
AN   1997-179255 [16]   WPIDS
DNC  C1997-057749
TI   Purifying active and infectious adenovirus - by contacting virus with
     chromatographic matrix material having binding gps. with affinity for
     virus confined to pores sufficiently large to allow virus to pass through
     undamaged.
DC   B04 D16
IN   ERICKSON, A E; ORIORDAN, C E; SMITH, A E; O'RIORDAN, C E; HELGERSON, A L
PA   (GENZ) GENZYME CORP; (HELG-I) HELGERSON A L; (ORIO-I) O'RIORDAN C E;
     (SMIT-I) SMITH A E
CYC  71
PI   WO 9708298      A1 19970306 (199716)* EN   62
        RW: AT BE CH DE DK EA ES FI FR GB GR IE IT KE LS LU MC MW NL OA PT SD
            SE SZ UG
         W: AL AM AT AU AZ BB BG BR BY CA CH CN CZ DE DK EE ES FI GB GE HU IL
            IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ
            PL PT RO RU SD SE SG SI SK TJ TM TR TT UA UG US
     AU 9670108      A  19970319 (199728)
     EP 847442       A1 19980617 (199828)  EN
         R: AL AT BE CH DE DK ES FI FR GB GR IE IT LI LT LU LV MC NL PT SE SI
     JP 11511326     W  19991005 (199953)       66
     AU 722196       B  20000727 (200041)
     US 6143548      A  20001107 (200059)
     US 2004152183   A1 20040805 (200452)
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ADT WO 9708298 A1 WO 1996-US13872 19960830; AU 9670108 A AU 1996-70108 19960830; EP 847442 A1 EP 1996-931425 19960830, WO 1996-US13872 19960830; JP 11511326 W WO 1996-US13872 19960830, JP 1997-510556 19960830; AU 722196 B AU 1996-70108 19960830; US 6143548 A Provisional US 1995-2967P 19950830, WO 1996-US13872 19960830, US 1998-11828 19980629; US 2004152183 A1 Provisional US 1995-2967P 19950830, Cont of WO 1996-US13872 19960830, Cont of US 1998-11828 19980629, Cont of US 2000-604349 20000627, US 2004-470604 20040302

FDT AU 9670108 A Based on WO 9708298; EP 847442 A1 Based on WO 9708298; JP 11511326 W Based on WO 9708298; AU 722196 B Previous Publ. AU 9670108, Based on WO 9708298; US 6143548 A Based on WO 9708298; US 2004152183 A1 Cont of US 6143548

PRAI US 1995-2967P 19950830; US 1998-11828 19980629; US 2000-604349 20000627; US 2004-470604 20040302

AB WO 9708298 A UPAB: 19970417

Purifying active and infectious adenovirus comprises contacting the virus with a chromatographic matrix material having binding gps. with affinity for the virus, which are confined to pores in the matrix sufficiently large to allow the virus to pass through undamaged. Also claimed are: (1) purifying active and infectious adenovirus comprising contacting the virus with a chromatographic matrix material comprising pores and having binding gps. with affinity for the virus, where the matrix further comprises crosslinking or tentacles sufficient to substantially prevent the virus from contacting the pores; and (2) purifying adeno-associated virus (AAV) from a sample also contg. adenovirus comprising contacting the sample with a chromatographic material capable of damaging adenovirus, where the damaged adenovirus becomes non-infectious.

USE - The methods are useful for the purificn. of commercially useful quantities of infectious adenovirus and AAV, esp. for therapeutic use, e.g. in gene transfer and gene therapy.

ADVANTAGE - The improved methods for contacting therapeutically useful viruses with suitable materials are used in chromatographic fractionation techniques in a fashion and under conditions so that viruses, esp. surface components believed to be required for infectivity, are not damaged by such contact.

Dwg.7AB/14

=> file uspatful

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	129.40	180.81

FILE 'USPATFULL' ENTERED AT 18:53:21 ON 22 JUN 2005
CA INDEXING COPYRIGHT (C) 2005 AMERICAN CHEMICAL SOCIETY (ACS)

FILE COVERS 1971 TO PATENT PUBLICATION DATE: 21 Jun 2005 (20050621/PD)
FILE LAST UPDATED: 21 Jun 2005 (20050621/ED)
HIGHEST GRANTED PATENT NUMBER: US6910221
HIGHEST APPLICATION PUBLICATION NUMBER: US2005132458
CA INDEXING IS CURRENT THROUGH 21 Jun 2005 (20050621/UPCA)
ISSUE CLASS FIELDS (/INCL) CURRENT THROUGH: 21 Jun 2005 (20050621/PD)
REVISED CLASS FIELDS (/NCL) LAST RELOADED: Apr 2005
USPTO MANUAL OF CLASSIFICATIONS THESAURUS ISSUE DATE: Apr 2005

>>> USPAT2 is now available. USPATFULL contains full text of the original, i.e., the earliest published granted patents or applications. USPAT2 contains full text of the latest US publications, starting in 2001, for the inventions covered in USPATFULL. A USPATFULL record contains not only the original published document but also a list of any subsequent publications. The publication number, patent kind code, and publication date for all the US publications for an invention are displayed in the PI (Patent Information) field of USPATFULL records and may be searched in standard search fields, e.g., /PN, /PK, etc. <<<

>>> USPATFULL and USPAT2 can be accessed and searched together through the new cluster USPATALL. Type FILE USPATALL to enter this cluster. <<<

>>> <<<

>>> Use USPATALL when searching terms such as patent assignees, classifications, or claims, that may potentially change from the earliest to the latest publication. <<<

This file contains CAS Registry Numbers for easy and accurate substance identification.

=> e vellekamp g j/in

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E1            1     VELLECO GUS/IN
E2            1     VELLEGO GENI/IN
E3            0 --> VELLEKAMP G J/IN
E4            4     VELLEKAMP GARY/IN
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E6            5     VELLEKOOP LINDA J/IN
E7            1     VELLEKOOP MARINUS H/IN
E8            3     VELLEKOOP MICHAEL JOHANNES/IN
E9            1     VELLEKOOP PIETER/IN
E10           1     VELLELLA VINCENT A/IN
E11           1     VELLEMAN KLAUS/IN
E12           1     VELLEMAN KOEN/IN

=> s e4 or e5
              4 "VELLEKAMP GARY"/IN
              3 "VELLEKAMP GARY J"/IN
L17           7 "VELLEKAMP GARY"/IN OR "VELLEKAMP GARY J"/IN

=> d l17,ti,1-7

L17  ANSWER 1 OF 7  USPATFULL on STN
TI     Compositions comprising viruses and methods for concentrating virus
       preparations

L17  ANSWER 2 OF 7  USPATFULL on STN
TI     Compostions comprising viruses and methods for concentrating virus
       preparations

L17  ANSWER 3 OF 7  USPATFULL on STN
TI     Method for purifying adenoviruses

L17  ANSWER 4 OF 7  USPATFULL on STN
TI     Methods for purifying viruses

L17  ANSWER 5 OF 7  USPATFULL on STN
TI     Methods for purifying viruses

L17  ANSWER 6 OF 7  USPATFULL on STN
TI     Purification of bacterially expressed human interleukin-10

L17  ANSWER 7 OF 7  USPATFULL on STN
TI     Purification of human interleukin-10 from a cell culture medium


=> d l17,cbib,clm,1-7

L17  ANSWER 1 OF 7  USPATFULL on STN
2003:225255 Compositions comprising viruses and methods for concentrating virus
    preparations.
    Frei, Andreas, Freehold, NJ, UNITED STATES
    Kwan, Henry K.H., Summit, NJ, UNITED STATES
    Sandweiss, Varda E., Forest Hills, NY, UNITED STATES
    Vellekamp, Gary J., Glen Ridge, NJ, UNITED STATES
    Yuen, Pui-Ho, Princeton Junction, NJ, UNITED STATES
    Bondoc, Laureano L., JR., Piscataway, NJ, UNITED STATES
    Porter, Frederick William, IV, Edison, NJ, UNITED STATES
    Tang, John Chu-Tay, Livingston, NJ, UNITED STATES
    Ihnat, Peter, Brooklyn, NY, UNITED STATES
    US 2003157066 A1 20030821
    APPLICATION: US 2003-365632  A1  20030404 (10)
    PRIORITY: US 1996-33176P  19961213 (60)
    US 1998-74873P  19980217 (60)
    US 1998-85559P  19980515 (60)
    DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM    What is claimed is:
       1. A composition comprising virus in a formulation comprising a
       polyhydroxy hydrocarbon buffered to maintain a pH in a range from about
       7 to about 8.5 at a temperature in the range from about 2° C. to
   •   27° C.

       2. The composition of claim 1 wherein the virus is a recombinant virus.

       3. The composition of claim 1 wherein the polyhydroxy hydrocarbon is
       glycerol.

       4. The composition of claim 1 further comprising a disaccharide.

       5. The composition of claim 1 wherein the composition comprises a buffer
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system comprising sodium phosphate monobasic dihydrate in a concentration of about 0.5 to 10 mg/mL and tromethamine in a concentration of about 0.5 to 10 mg/mL.

6. The composition of claim 1 further comprising a divalent metal salt in a concentration of about 0.1 to 1 mg/mL.

7. The composition of claim 1 which further comprises a diluent comprising water.

8. The composition of claim 1 wherein the virus is present in a concentration of about 1×10^{9} to 1×10^{13} particles/mL.

9. The composition of claim 1 wherein the virus is adenovirus.

10. The composition of claim 2 wherein the recombinant virus comprises a wild-type p53 gene.

11. The composition of claim 10 wherein the recombinant virus is A/C/N153.

12. The composition of claim 1 wherein the virus is adenovirus; the polyhydroxy hydrocarbon is glycerol; the buffer system maintains the pH in a range from about 7.3 to about 7.9 at a temperature ranging from 20° C. to 27° C.; and the composition further comprises a disaccharide.

13. The composition of claim 12 wherein the adenovirus is A/C/N/53; the disaccharide is sucrose; the buffer system comprises sodium phosphate monobasic dihydrate and tromethamine; and the composition further comprises a divalent metal salt and water.

14. A method for concentrating a virus preparation comprising: (a) adding a polyhydroxy hydrocarbon to a virus preparation to a final polyhydroxy hydrocarbon concentration of about 20% or more; and (b) subjecting the virus preparation to a filtration process wherein the concentration of virus is increased by applying pressure to the preparation such that diluent is removed from the virus preparation through a filter while the virus is retained.

15. The method of claim 14 wherein the filtration process comprises ultrafiltration.

16. The method of claim 14 wherein the filtration process comprises tangential flow filtration.

17. A method of purifying a virus preparation comprising: (a) subjecting the virus preparation to anion-exchange chromatography, wherein the virus is eluted as a virus preparation product from an anion-exchange chromatographic medium; (b) adding a polyhydroxy hydrocarbon to the virus preparation product of step (a) so that the concentration of polyhydroxy hydrocarbon in the preparation reaches a level of about 25% or more; and (c) increasing the concentration of virus in the virus preparation product of step (b) by applying pressure to the preparation such that diluent is removed from the virus preparation through a filter while the virus is retained; and (d) subjecting the concentrated virus preparation product of step (c) to one or more additional processing steps.

18. The method of claim 14 wherein the virus is a recombinant virus.

19. The method of claim 14 wherein the virus is a recombinant virus carrying a therapeutic transgene for use in gene therapy.

20. The method of claim 17 wherein an additional processing step comprises size exclusion chromatography.

21. The method of claim 17 wherein the process of step (c) comprises tangential flow filtration.

22. The method of claim 21 wherein the process of step (c) is carried out using apparatus comprising a Pellicon XL filtration system.

23. A virus preparation concentrated by the method of claim 14.

24. A virus preparation purified by the method of claim 17.

25. A method for concentrating a virus preparation comprising: (a)

centrifuging a composition which comprises a first layer comprising a polyhydroxy hydrocarbon in a concentration of 35% to 80% (v/v), the first layer overlaid with a second layer comprising a polyhydroxy hydrocarbon in a concentration of 5% to 30% (v/v), the second layer overlaid with a third layer comprising virus; and (b) recovering the virus from the first layer.

26. The composition of claim 1, wherein the composition is subjected to an additional processing step of agitation, followed by microfiltration, for the prevention of particulate formation during storage of the composition.

27. The composition of claim 1, wherein the composition is subjected to one or more freeze/thaw cycles, followed by agitation, and then microfiltration, for the prevention of particulate formation during storage of the composition.

28. The composition of claim 12 further comprising a monovalent metal salt in a concentration of about 0.6 to 10.0 mg/mL.

L17 ANSWER 2 OF 7 USPATFULL on STN
2003:95964 Compostions comprising viruses and methods for concentrating virus preparations.
Frei, Andreas, Freehold, NJ, United States
Kwan, Henry K. H., Summit, NJ, United States
Sandweiss, Varda E., Forest Hills, NY, United States
Vellekamp, Gary J., Glen Ridge, NJ, United States
Yuen, Pui-Ho, Princeton Junction, NJ, United States
Ihnat, Peter, Brooklyn, NY, United States
Schering Corporation, Kenilworth, NJ, United States (U.S. corporation)
US 6544769 B1 20030408
APPLICATION: US 1999-249646 19990212 (9)
PRIORITY: US 1998-85559P 19980515 (60)
US 1998-74873P 19980217 (60)
US 1996-33176P 19961213 (60)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:
1. A composition comprising a recombinant adenovirus, 20 to 200 mg/ml glycerol, and an aqueous buffer system which maintains a pH range from about 7 to about 8.5 at a temperature range from about 2° C. to 27° C., wherein the recombinant adenovirus comprises a wild-type p53 gene.

2. The composition of claim 1 wherein the recombinant adenovirus is A/C/N/53.

3. The composition of claim 2 which further comprises sucrose and a divalent metal salt, and wherein the aqueous buffer system comprises sodium phosphate monobasic dihydrate and tromethamine and maintains a pH range from about 7.3 to about 7.9.

4. A composition comprising adenovirus, 20 to 200 mg/ml glycerol, sucrose, and an aqueous buffer system which maintains a pH range from about 7 to about 8.5 at a temperature range from about 2° C. to 27° C.

5. A composition comprising from 1×10^{9} to 1×10^{13} particles of A/C/N/53, 20 to 200 mg/ml glycerol, 5 to 25 mg/ml sucrose, a divalent metal salt, and an aqueous buffer system which comprises from 0.5 to 10 mg/ml sodium phosphate monobasic dihydrate and from 0.5 to 10 mg/ml tromethamine and which maintains a pH range from 7.3 to 8 at a temperature range from 4° C. to 27° C.

L17 ANSWER 3 OF 7 USPATFULL on STN
2002:126348 Method for purifying adenoviruses.
Cannon-Carlson, Susan V., Wayne, NJ, UNITED STATES
Cutler, Collette, Bloomingdale, NJ, UNITED STATES
Vellekamp, Gary J., Glen Ridge, NJ, UNITED STATES
Voloch, Marcio, New York, NY, UNITED STATES
Schering Corporation (U.S. corporation)
US 2002064860 A1 20020530
APPLICATION: US 2001-991080 A1 20011116 (9)
PRIORITY: US 2000-253823P 20001129 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:

1. A method for purifying adenovirus from contaminants in a sample pool, comprising: contacting the sample pool with a hydroxyapatite chromatographic medium to reversibly bind the adenovirus to the hydroxyapatite; and eluting the bound adenovirus from the hydroxyapatite.

2. The method of claim 1, wherein the sample pool comprises sodium chloride in a concentration of from about 150 to 500 mM.

3. The method of claim 1, wherein the hydroxyapatite chromatographic medium is equilibrated with a buffer comprising sodium chloride at a concentration of from about 150 to 500 mM before the step of contacting the sample pool with the hydroxyapatite.

4. The method of claim 1, further comprising the step of washing the hydroxyapatite with a buffer comprising sodium chloride in a concentration of 150 to 500 mM, wherein the hydroxyapatite comprises an adenovirus bound thereto.

5. The method of claim 1, wherein the adenovirus is eluted using a buffer comprising sodium chloride in a concentration from about 150 mM to 500 mM.

6. The method of claim 1 wherein the sodium chloride concentration in a buffer used in the method is from about 350 mM to 450 mM.

7. The method of claim 1, wherein the sample pool is prepared from an eluate of a conventional chromatography medium.

8. The method of claim 7 wherein the conventional chromatography medium is: an anion exchange resin; an immobilized metal ion affinity resin; a size exclusion chromatography resin; or a medium used in hydrophobic interaction chromatography.

9. The method of claim 1, wherein the eluting step is a gradient elution to 600 mM phosphate.

10. The method of claim 1, wherein the eluting step is a step elution with 250 mM phosphate.

11. The method of claim 1, wherein a buffer used in the method comprises glycerol or sucrose.

12. The method of claim 1, wherein the concentration of adenovirus in the sample pool is equal to or less than 1×10^{14} particles per ml.

13. The method of claim 1, wherein the adenovirus comprises a therapeutic gene.

14. The method of claim 1, wherein the adenovirus is ACN53.

15. The method of claim 1, wherein the adenovirus comprises a nucleic acid sequence from the p53 gene or from the p21 gene.

16. A method of claim 1, which reduces the concentration of a contaminant in the sample pool by at least 80%.

17. A method of claim 1, which reduces the concentration of empty capsids by at least 75%.

18. A method of claim 1, which reduces the concentration of BSA by at least 70%.

19. A method for purifying adenovirus from contaminants in sample pool, comprising: contacting the sample pool with a hydroxyapatite chromatographic medium to reversibly bind the adenovirus to the hydroxyapatite; washing the adenovirus-bound hydroxyapatite with a buffered solution; and eluting the bound adenovirus from the hydroxyapatite, wherein the sample pool is a buffered solution comprising about 50 mM sodium phosphate pH about 7.5, about 400 mM sodium chloride, about 2% sucrose, about 2 mM $MgCl_2$, and about 10% glycerol, and the concentration of total contaminants is reduced by at least 80%.

L17 ANSWER 4 OF 7 USPATFULL on STN
2001:194143 Methods for purifying viruses.
Tang, John Chu-Tay, Livingston, NJ, United States

Vellekamp, Gary, Glen Ridge, NJ, United States
Bondoc, Laureano L., JR., Piscataway, NJ, United States
US 2001036657 A1 20011101
APPLICATION: US 2001-872134 A1 20010601 (9)
PRIORITY: US 1996-33176P 19961213 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:

1. A method for purification of a virus preparation comprising: a) subjecting the virus preparation to anion-exchange chromatography, wherein the virus is eluted from an anion-exchange chromatographic medium; and b) subjecting the virus product of step A to size exclusion chromatography, wherein the virus is eluted from a size exclusion chromatographic medium.

2. The method of claim 1, wherein the virus preparation is a cell lysate.

3. The method of claim 2, wherein the cell lysate is filtered before step a.

4. The method of claim 1, wherein the virus is a recombinant adenovirus.

5. The method of claim 1, wherein the anion-exchange medium is FRACTOGEL.TM.-DEAE.

6. The method of claim 1, wherein the size exclusion medium is Superdex-200.

7. The method of claim 1, wherein the size-exclusion medium is provided in a column prepared as a salt gradient decreasing in ionic strength from the top of the column towards the bottom, the top of the column having a buffer having an ionic strength substantially identical to that of the product of step a.

8. The method of claim 1 wherein the anion exchange medium comprises diethylaminoethyl groups on a cross-linked agarose, cellulose, polyacrylamide or polystyrene backbone.

9. The method of claim 1, wherein the size-exclusion medium comprises a cross-linked polysaccharide.

10. The method of claim 9, wherein the cross-linked polysaccharide is a composite of cross-linked agarose and dextran.

17. The method of claim 1 wherein the virus is ACN53.

18. The method of claim 1, wherein the anion exchange chromatographic medium is extensively washed before application of the virus preparation.

19. A virus purified by the method of claim 1.

L17 ANSWER 5 OF 7 USPATFULL on STN
2001:112091 Methods for purifying viruses.
Tang, John Chu-Tay, Livingston, NJ, United States
Vellekamp, Gary, Glen Ridge, NJ, United States
Bondoc, Jr., Laureano L., Piscataway, NJ, United States
Schering Corporation, Kenilworth, NJ, United States (U.S. corporation)
US 6261823 B1 20010717
APPLICATION: US 1997-989227 19971211 (8)
PRIORITY: US 1996-33176P 19961213 (60)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:

1. A method of purifying adenovirus from a virus preparation, comprising the successive steps of: a) subjecting the virus preparation to anion-exchange chromatography, wherein the adenovirus is eluted from an anion-exchange chromatographic medium; and b) subjecting the eluate of step a) which contains the adenovirus to size exclusion chromatography, wherein the adenovirus is eluted from a size exclusion chromatographic medium.

2. The method of claim 1, wherein the virus preparation is a cell lysate.

3. The method of claim 2, wherein the cell lysate is filtered before step a).

4. The method of claim 1, wherein the adenovirus is recombinant.

5. The method of claim 1 wherein the adenovirus is ACN53.

6. The method of claim 1, wherein the anion-exchange medium is DEAE-FRACTOGEL.

7. The method of claim 1, wherein the size exclusion medium is SUPERDEX-200.

8. The method of claim 1 wherein the anion-exchange medium comprises diethylaminoethyl groups on a cross-linked agarose, cellulose, polyacrylamide or polystyrene backbone.

9. The method of claim 1, wherein the size-exclusion medium comprises a cross-linked polysaccharide.

10. The method of claim 9, wherein the cross-linked polysaccharide is a composite of cross-linked agarose and dextran.

11. The method of claim 1, wherein the anion-exchange chromatographic medium is extensively washed before step a).

12. The method of claim 1, wherein step b) further comprises eluting the adenovirus from the size-exclusion chromatographic medium into a low-salt buffer by a high-salt elution buffer, wherein the size-exclusion medium is in a column containing a salt gradient which decreases in ionic strength from the top of the column towards the bottom of the column.

13. The method of claim 1, wherein the anion-exchange chromatographic medium or the size exclusion chromatographic medium is contacted with a buffer comprising glycerol, including wash, equilibration, loading and elution buffer.

14. The method of claim 1, further comprising a step of adding glycerol to a fraction which contains the adenovirus.

L17 ANSWER 6 OF 7 USPATFULL on STN
1998:7167 Purification of bacterially expressed human interleukin-10.
Vellekamp, Gary, Glen Ridge, NJ, United States
Cannon-Carlson, Susan, Wayne, NJ, United States
Tang, John, Livingston, NJ, United States
Schering Corporation, Kenilworth, NJ, United States (U.S. corporation)
US 5710251 19980120
WO 9420525 19940915
APPLICATION: US 1995-495558 19951218 (8)
WO 1994-US1909 19940303 19951218 PCT 371 date 19951218 PCT 102(e) date
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed:
1. A method for purifying bacterially expressed human Interleukin-10 (IL-10) contained within a solution comprising: (a) applying a solution containing IL-10 to a cation exchange chromatography column thereby obtaining fractions containing IL-10; (b) applying the IL-10-containing fractions from step (a) to an anion exchange chromatography column thereby obtaining fractions containing IL-10; (c) applying the IL-10-containing fractions from step (b) to a hydroxyapatite chromatography chromatography thereby obtaining fractions containing a single isolated dimer of IL-10.

2. The method of claim 1 wherein the cation exchange chromatography column from step (a) is comprised of sulfonate exchange groups attached to a support matrix.

3. The method of claim 2 wherein the support matrix is agarose.

4. The method of claim 1 wherein the anion exchange chromatography column is comprised of quaternary amino ethyl exchange groups attached to a support matrix.

5. The method of claim 4 wherein the support matrix is agarose.

6. The method of claim 1 further comprising applying the IL-10-containing fractions obtained from step (c) of claim 1 to a gel filtration chromatography column to obtain dimeric IL-10 substantially free of high and low molecular weight impurities.

7. The method of claim 6 wherein the material in the gel filtration chromatography column has a fractionation range of from 1 to 600 kDa.

8. The method of claim 1 wherein prior to step (a) the bacterially expressed human IL-10 is extracted from bacteria in inclusion bodies, denatured and refolded into biologically active human IL-10.

9. A method for separating non-acetylated homodimers of Interleukin-10 (IL-10) from acetylated IL-10 homodimers and from acetylated IL-10 heterodimers contained within a solution comprising: applying the solution to an anion exchange chromatography column under conditions in which the non-acetylated homodimers are separated from the acetylated homodimers and from the acetylated heterodimers.

L17 ANSWER 7 OF 7 USPATFULL on STN
94:60238 Purification of human interleukin-10 from a cell culture medium.
Vellekamp, Gary, Glen Ridge, NJ, United States
Cannon-Carlson, Susan, Wayne, NJ, United States
Tang, John, Livingston, NJ, United States
Schering-Plough, Kenilworth, NJ, United States (U.S. corporation)
US 5328989 19940712
APPLICATION: US 1993-26942 19930305 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:
1. A method for purifying Interleukin-10 (IL-10) from a cell culture medium comprising: (a) subjecting the culture medium containing IL-10 to cation exchange chromatography thereby obtaining fractions containing IL-10; (b) subjecting the IL-10-containing fractions from step (a) to anion exchange chromatography thereby obtaining fractions containing IL-10; (c) subjecting the IL-10-containing fractions from step (b) to hydroxyapatite chromatography thereby obtaining fractions containing a single isolated dimer of IL-10.

2. The method of claim 1 wherein the cation exchange chromatography from step (a) uses a column comprised of sulfonate exchange groups attached to a support matrix.

3. The method of claim 2 wherein the support matrix is agarose.

4. The method of claim 1 wherein the anion exchange chromatography uses a column comprised of quartenary amino ethyl exchange groups attached to a support matrix.

5. The method of claim 4 wherein the support matrix is agarose.

6. The method of claim 1 further comprising applying the IL-10-containing fractions obtained from step (c) of claim 1 to a gel filtration chromatography column to obtain dimeric IL-10 substantially free of high and low molecular weight impurities.

7. The method of claim 6 wherein the gel has a fractionation range of from 1 to 600 kDa.

8. The method of claim 1 wherein the IL-10 is human IL-10.

9. The method of claim 1 wherein IL-10 is secreted in a cell culture medium from eukaryotic cell.

10. A method for separating different dimers of IL-10 present in an IL-10 containing protein fraction comprising: subjecting the IL-10 containing fraction to hydroxyapatite chromatography under conditions wherein the different IL-10 dimers are separated from each other.

11. The method of claim 10 wherein the IL-10 dimers present in the protein fraction are $\Delta 0{:}\Delta 0$, $\Delta 0{:}\Delta 2$ and $\Delta 2{:}\Delta 2$ IL-10 dimers.

12. The method of claim 11 wherein the IL-10 dimer which is collected is the $\Delta 0{:}\Delta 0$ IL-10 dimer.

13. The method of claim 11 wherein the IL-10 dimer which is collected is the $\Delta 0{:}\Delta 2$ IL-10 dimer.

14. The method of claim 11 wherein the IL-10 dimer which is collected is the $\Delta 2{:}\Delta 2$ IL-10 dimer.

15. The method of claim 10 wherein the hydroxyapatite is sintered hydroxyapatite.

16. A method for separating different dimers of a protein contained within a protein fraction wherein the different dimers have different N-terminal amino acid sequences comprising: subjecting the protein fraction to hydroxyapatite chromatography under conditions wherein the different dimers of the protein are separated from each other.

17. A method for separating variants of a protein contained within a protein fraction wherein the variants of the protein have different N-terminal amino acid sequences comprising: subjecting the protein fraction to hydroxyapatite chromatography under conditions wherein the variants of the protein are separated from each other.

=> s l17 and hydroxyapatite
9532 HYDROXYAPATITE
L18 3 L17 AND HYDROXYAPATITE

=> d l18,cbib,clm,1-3

L18 ANSWER 1 OF 3 USPATFULL on STN
2002:126348 Method for purifying adenoviruses.
Cannon-Carlson, Susan V., Wayne, NJ, UNITED STATES
Cutler, Collette, Bloomingdale, NJ, UNITED STATES
Vellekamp, Gary J., Glen Ridge, NJ, UNITED STATES
Voloch, Marcio, New York, NY, UNITED STATES
Schering Corporation (U.S. corporation)
US 2002064860 A1 20020530
APPLICATION: US 2001-991080 A1 20011116 (9)
PRIORITY: US 2000-253823P 20001129 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:
1. A method for purifying adenovirus from contaminants in a sample pool, comprising: contacting the sample pool with a **hydroxyapatite** chromatographic medium to reversibly bind the adenovirus to the **hydroxyapatite**; and eluting the bound adenovirus from the **hydroxyapatite**.

2. The method of claim 1, wherein the sample pool comprises sodium chloride in a concentration of from about 150 to 500 mM.

3. The method of claim 1, wherein the **hydroxyapatite** chromatographic medium is equilibrated with a buffer comprising sodium chloride at a concentration of from about 150 to 500 mM before the step of contacting the sample pool with the **hydroxyapatite**.

4. The method of claim 1, further comprising the step of washing the **hydroxyapatite** with a buffer comprising sodium chloride in a concentration of 150 to 500 mM, wherein the **hydroxyapatite** comprises an adenovirus bound thereto.

5. The method of claim 1, wherein the adenovirus is eluted using a buffer comprising sodium chloride in a concentration from about 150 mM to 500 mM.

6. The method of claim 1 wherein the sodium chloride concentration in a buffer used in the method is from about 350 mM to 450 mM.

7. The method of claim 1, wherein the sample pool is prepared from an eluate of a conventional chromatography medium.

8. The method of claim 7 wherein the conventional chromatography medium is: an anion exchange resin; an immobilized metal ion affinity resin; a size exclusion chromatography resin; or a medium used in hydrophobic interaction chromatography.

9. The method of claim 1, wherein the eluting step is a gradient elution to 600 mM phosphate.

10. The method of claim 1, wherein the eluting step is a step elution with 250 mM phosphate.

11. The method of claim 1, wherein a buffer used in the method comprises glycerol or sucrose.

12. The method of claim 1, wherein the concentration of adenovirus in the sample pool is equal to or less than 1×10^{14} particles per ml.

13. The method of claim 1, wherein the adenovirus comprises a therapeutic gene.

14. The method of claim 1, wherein the adenovirus is ACN53.

15. The method of claim 1, wherein the adenovirus comprises a nucleic acid sequence from the p53 gene or from the p21 gene.

16. A method of claim 1, which reduces the concentration of a contaminant in the sample pool by at least 80%.

17. A method of claim 1, which reduces the concentration of empty capsids by at least 75%.

18. A method of claim 1, which reduces the concentration of BSA by at least 70%.

19. A method for purifying adenovirus from contaminants in sample pool, comprising: contacting the sample pool with a **hydroxyapatite** chromatographic medium to reversibly bind the adenovirus to the **hydroxyapatite**; washing the adenovirus-bound **hydroxyapatite** with a buffered solution; and eluting the bound adenovirus from the **hydroxyapatite**, wherein the sample pool is a buffered solution comprising about 50 mM sodium phosphate pH about 7.5, about 400 mM sodium chloride, about 2% sucrose, about 2 mM $MgCl_2$, and about 10% glycerol, and the concentration of total contaminants is reduced by at least 80%.

L18 ANSWER 2 OF 3 USPATFULL on STN
1998:7167 Purification of bacterially expressed human interleukin-10.
Vellekamp, Gary, Glen Ridge, NJ, United States
Cannon-Carlson, Susan, Wayne, NJ, United States
Tang, John, Livingston, NJ, United States
Schering Corporation, Kenilworth, NJ, United States (U.S. corporation)
US 5710251 19980120
WO 9420525 19940915
APPLICATION: US 1995-495558 19951218 (8)
WO 1994-US1909 19940303 19951218 PCT 371 date 19951218 PCT 102(e) date
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:
1. A method for purifying bacterially expressed human Interleukin-10 (IL-10) contained within a solution comprising: (a) applying a solution containing IL-10 to a cation exchange chromatography column thereby obtaining fractions containing IL-10; (b) applying the IL-10-containing fractions from step (a) to an anion exchange chromatography column thereby obtaining fractions containing IL-10; (c) applying the IL-10-containing fractions from step (b) to a **hydroxyapatite** chromatography chromatography thereby obtaining fractions containing a single isolated dimer of IL-10.

2. The method of claim 1 wherein the cation exchange chromatography column from step (a) is comprised of sulfonate exchange groups attached to a support matrix.

3. The method of claim 2 wherein the support matrix is agarose.

4. The method of claim 1 wherein the anion exchange chromatography column is comprised of quaternary amino ethyl exchange groups attached to a support matrix.

5. The method of claim 4 wherein the support matrix is agarose.

6. The method of claim 1 further comprising applying the IL-10-containing fractions obtained from step (c) of claim 1 to a gel filtration chromatography column to obtain dimeric IL-10 substantially free of high and low molecular weight impurities.

7. The method of claim 6 wherein the material in the gel filtration chromatography column has a fractionation range of from 1 to 600 kDa.

8. The method of claim 1 wherein prior to step (a) the bacterially expressed human IL-10 is extracted from bacteria in inclusion bodies,

denatured and refolded into biologically active human IL-10.

9. A method for separating non-acetylated homodimers of Interleukin-10 (IL-10) from acetylated IL-10 homodimers and from acetylated IL-10 heterodimers contained within a solution comprising: applying the solution to an anion exchange chromatography column under conditions in which the non-acetylated homodimers are separated from the acetylated homodimers and from the acetylated heterodimers.

L18 ANSWER 3 OF 3 USPATFULL on STN
94:60238 Purification of human interleukin-10 from a cell culture medium.
Vellekamp, Gary, Glen Ridge, NJ, United States
Cannon-Carlson, Susan, Wayne, NJ, United States
Tang, John, Livingston, NJ, United States
Schering-Plough, Kenilworth, NJ, United States (U.S. corporation)
US 5328989 19940712
APPLICATION: US 1993-26942 19930305 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:
1. A method for purifying Interleukin-10 (IL-10) from a cell culture medium comprising: (a) subjecting the culture medium containing IL-10 to cation exchange chromatography thereby obtaining fractions containing IL-10; (b) subjecting the IL-10-containing fractions from step (a) to anion exchange chromatography thereby obtaining fractions containing IL-10; (c) subjecting the IL-10-containing fractions from step (b) to **hydroxyapatite** chromatography thereby obtaining fractions containing a single isolated dimer of IL-10.

2. The method of claim 1 wherein the cation exchange chromatography from step (a) uses a column comprised of sulfonate exchange groups attached to a support matrix.

3. The method of claim 2 wherein the support matrix is agarose.

4. The method of claim 1 wherein the anion exchange chromatography uses a column comprised of quartenary amino ethyl exchange groups attached to a support matrix.

5. The method of claim 4 wherein the support matrix is agarose.

6. The method of claim 1 further comprising applying the IL-10-containing fractions obtained from step (c) of claim 1 to a gel filtration chromatography column to obtain dimeric IL-10 substantially free of high and low molecular weight impurities.

7. The method of claim 6 wherein the gel has a fractionation range of from 1 to 600 kDa.

8. The method of claim 1 wherein the IL-10 is human IL-10.

9. The method of claim 1 wherein IL-10 is secreted in a cell culture medium from eukaryotic cell.

10. A method for separating different dimers of IL-10 present in an IL-10 containing protein fraction comprising: subjecting the IL-10 containing fraction to **hydroxyapatite** chromatography under conditions wherein the different IL-10 dimers are separated from each other.

11. The method of claim 10 wherein the IL-10 dimers present in the protein fraction are $\Delta 0{:}\Delta 0$, $\Delta 0{:}\Delta 2$ and $\Delta 2{:}\Delta 2$ IL-10 dimers.

12. The method of claim 11 wherein the IL-10 dimer which is collected is the $\Delta 0{:}\Delta 0$ IL-10 dimer.

13. The method of claim 11 wherein the IL-10 dimer which is collected is the $\Delta 0{:}\Delta 2$ IL-10 dimer.

14. The method of claim 11 wherein the IL-10 dimer which is collected is the $\Delta 2{:}\Delta 2$ IL-10 dimer.

15. The method of claim 10 wherein the **hydroxyapatite** is sintered **hydroxyapatite**.

16. A method for separating different dimers of a protein contained within a protein fraction wherein the different dimers have different

N-terminal amino acid sequences comprising: subjecting the protein fraction to **hydroxyapatite** chromatography under conditions wherein the different dimers of the protein are separated from each other.

17. A method for separating variants of a protein contained within a protein fraction wherein the variants of the protein have different N-terminal amino acid sequences comprising: subjecting the protein fraction to **hydroxyapatite** chromatography under conditions wherein the variants of the protein are separated from each other.

```
=> e cutler collette/in
E1            1     CUTLER CHRISTOPHER W/IN
E2            1     CUTLER CLIFFORD P/IN
E3            1 --> CUTLER COLLETTE/IN
E4            2     CUTLER DAN/IN
E5            1     CUTLER DANIEL JAMES/IN
E6            1     CUTLER DANIEL S/IN
E7            1     CUTLER DANNY L/IN
E8            1     CUTLER DAVE/IN
E9            1     CUTLER DAVE WILLIAM/IN
E10           5     CUTLER DAVID/IN
E11           1     CUTLER DAVID G/IN
E12           2     CUTLER DAVID J/IN

=> s e3
L19           1 "CUTLER COLLETTE"/IN

=> d l19,cbib

L19  ANSWER 1 OF 1  USPATFULL on STN
2002:126348 Method for purifying adenoviruses.
    Cannon-Carlson, Susan V., Wayne, NJ, UNITED STATES
    Cutler, Collette, Bloomingdale, NJ, UNITED STATES
    Vellekamp, Gary J., Glen Ridge, NJ, UNITED STATES
    Voloch, Marcio, New York, NY, UNITED STATES
    Schering Corporation (U.S. corporation)
    US 2002064860 A1 20020530
    APPLICATION: US 2001-991080  A1  20011116 (9)
    PRIORITY: US 2000-253823P  20001129 (60)
    DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.


=> e cannon-carlson s v/in
E1            3     CANNON WILLIAM P/IN
E2            1     CANNON WILLIAM R/IN
E3            0 --> CANNON-CARLSON S V/IN
E4            3     CANNONE ANTHONY G/IN
E5            4     CANNONE DOMENICO/IN
E6            1     CANNONE GREGORY/IN
E7            2     CANNONE GREGORY M/IN
E8            1     CANNONE ROBERT P/IN
E9            1     CANNONE SALVATORE L/IN
E10           1     CANNONS ANDREW/IN
E11           1     CANNONS ANDREW CLIVE/IN
E12           1     CANNOVA CHRISTINE L/IN

=> e cannon carlson s v/in
E1            3     CANNON BRET D/IN
E2            7     CANNON BRUCE L/IN
E3            0 --> CANNON CARLSON S V/IN
E4            3     CANNON CARLSON SUSAN/IN
E5            2     CANNON CARLSON SUSAN V/IN
E6            3     CANNON CARTER/IN
E7            6     CANNON CARTER S/IN
E8            1     CANNON CARTER SCOTT/IN
E9            1     CANNON CHARLES/IN
E10           1     CANNON CHARLES C/IN
E11           3     CANNON CHARLES E/IN
E12           1     CANNON CHARLES G/IN

=> s e4 or e5
              3 "CANNON CARLSON SUSAN"/IN
              2 "CANNON CARLSON SUSAN V"/IN
L20           5 "CANNON CARLSON SUSAN"/IN OR "CANNON CARLSON SUSAN V"/IN

=> d his
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     (FILE 'HOME' ENTERED AT 18:36:36 ON 22 JUN 2005)

     FILE 'USPATFULL' ENTERED AT 18:36:44 ON 22 JUN 2005
L1          9532 S HYDROXYAPATITE
L2          1599 S L1 AND HYDROXYAPATITE/CLM
L3            69 S L2 AND ADENOVIR?
L4            13 S L3 AND ADENOVIR?/CLM
L5            33 S L3 AND AY<2001
L6            27 S L5 NOT L4
L7             1 S US5496926/PN
L8             1 S L7 AND ADENOVIR?
L9             1 S US5624833/PN
L10            1 S L9 AND ADENOVIR?
               E ERICKSON AMY E/IN
L11            1 S E3
               E SHABRAM PAUL W/IN
L12            5 S E3

     FILE 'WPIDS' ENTERED AT 18:46:16 ON 22 JUN 2005
L13         2918 S HYDROXYAPATITE
L14           14 S L13 AND ADENOVIR?
               E SHABRAM P W/IN
L15            6 S E3
               E ERICKSON A E/IN
L16            1 S E3

     FILE 'USPATFULL' ENTERED AT 18:53:21 ON 22 JUN 2005
               E VELLEKAMP G J/IN
L17            7 S E4 OR E5
L18            3 S L17 AND HYDROXYAPATITE
               E CUTLER COLLETTE/IN
L19            1 S E3
               E CANNON-CARLSON S V/IN
               E CANNON CARLSON S V/IN
L20            5 S E4 OR E5

=> d 120,bib,clm,1-5

L20  ANSWER 1 OF 5  USPATFULL on STN
Full Text
AN     2002:126348  USPATFULL
TI     Method for purifying adenoviruses
IN     Cannon-Carlson, Susan V., Wayne, NJ, UNITED STATES
       Cutler, Collette, Bloomingdale, NJ, UNITED STATES
       Vellekamp, Gary J., Glen Ridge, NJ, UNITED STATES
       Voloch, Marcio, New York, NY, UNITED STATES
PA     Schering Corporation (U.S. corporation)
PI     US 2002064860      A1   20020530
AI     US 2001-991080     A1   20011116 (9)
PRAI   US 2000-253823P     20001129 (60)
DT     Utility
FS     APPLICATION
LREP   SCHERING-PLOUGH CORPORATION, PATENT DEPARTMENT (K-6-1, 1990), 2000
       GALLOPING HILL ROAD, KENILWORTH, NJ, 07033-0530
CLMN   Number of Claims: 19
ECL    Exemplary Claim: 1
DRWN   No Drawings
LN.CNT 423
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM    What is claimed is:
       1. A method for purifying adenovirus from contaminants in a sample pool,
       comprising:  contacting the sample pool with a hydroxyapatite
       chromatographic medium to reversibly bind the adenovirus to the
       hydroxyapatite; and  eluting the bound adenovirus from the
       hydroxyapatite.

       2. The method of claim 1, wherein the sample pool comprises sodium
       chloride in a concentration of from about 150 to 500 mM.

       3. The method of claim 1, wherein the hydroxyapatite chromatographic
       medium is equilibrated with a buffer comprising sodium chloride at a
       concentration of from about 150 to 500 mM before the step of contacting
       the sample pool with the hydroxyapatite.

       4. The method of claim 1, further comprising the step of washing the
       hydroxyapatite with a buffer comprising sodium chloride in a
       concentration of 150 to 500 mM, wherein the hydroxyapatite comprises an
       adenovirus bound thereto.
```

5. The method of claim 1, wherein the adenovirus is eluted using a buffer comprising sodium chloride in a concentration from about 150 mM to 500 mM.

6. The method of claim 1 wherein the sodium chloride concentration in a buffer used in the method is from about 350 mM to 450 mM.

7. The method of claim 1, wherein the sample pool is prepared from an eluate of a conventional chromatography medium.

8. The method of claim 7 wherein the conventional chromatography medium is: an anion exchange resin; an immobilized metal ion affinity resin; a size exclusion chromatography resin; or a medium used in hydrophobic interaction chromatography.

9. The method of claim 1, wherein the eluting step is a gradient elution to 600 mM phosphate.

10. The method of claim 1, wherein the eluting step is a step elution with 250 mM phosphate.

11. The method of claim 1, wherein a buffer used in the method comprises glycerol or sucrose.

12. The method of claim 1, wherein the concentration of adenovirus in the sample pool is equal to or less than 1×10^{14} particles per ml.

13. The method of claim 1, wherein the adenovirus comprises a therapeutic gene.

14. The method of claim 1, wherein the adenovirus is ACN53.

15. The method of claim 1, wherein the adenovirus comprises a nucleic acid sequence from the p53 gene or from the p21 gene.

16. A method of claim 1, which reduces the concentration of a contaminant in the sample pool by at least 80%.

17. A method of claim 1, which reduces the concentration of empty capsids by at least 75%.

18. A method of claim 1, which reduces the concentration of BSA by at least 70%.

19. A method for purifying adenovirus from contaminants in sample pool, comprising: contacting the sample pool with a hydroxyapatite chromatographic medium to reversibly bind the adenovirus to the hydroxyapatite; washing the adenovirus-bound hydroxyapatite with a buffered solution; and eluting the bound adenovirus from the hydroxyapatite, wherein the sample pool is a buffered solution comprising about 50 mM sodium phosphate pH about 7.5, about 400 mM sodium chloride, about 2% sucrose, about 2 mM $MgCl_2$, and about 10% glycerol, and the concentration of total contaminants is reduced by at least 80%.

L20 ANSWER 2 OF 5 USPATFULL on STN
Full Text
AN 2002:84882 USPATFULL
TI Pegylated interleukin-10
IN Lee, Seoju, Edison, NJ, UNITED STATES
Wylie, David C., Cranford, NJ, UNITED STATES
Cannon-Carlson, Susan V., Wayne, NJ, UNITED STATES
PI US 2002044921 A1 20020418
AI US 2001-967223 A1 20010928 (9)
PRAI US 2000-236596P 20000929 (60)
DT Utility
FS APPLICATION
LREP SCHERING-PLOUGH CORPORATION, PATENT DEPARTMENT (K-6-1, 1990), 2000 GALLOPING HILL ROAD, KENILWORTH, NJ, 07033-0530
CLMN Number of Claims: 20
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 847
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:
1. A mono-PEG-IL-10.

2. The mono-PEG-IL-10 of claim 1, comprising one or two PEG molecules covalently attached via a linker to one amino acid residue on IL-10, wherein the attachment is at an N-terminal amino acid residue or on a lysine residue.

3. The mono-PEG-IL-10 of claim 2: (a) which comprises a methoxy PEG; (b) wherein the IL-10 is human IL-10; (c) wherein the total molecular mass of all PEG covalently attached to the linker is from 3,000 daltons to 60,000 daltons; or (d) wherein the linker is a linear or branched C_{1-11} alkyl.

4. The mono-PEG-IL-10 of claim 2, wherein the total molecular mass of all PEG covalently attached to the linker is from 10,000 daltons to 36,000 daltons.

5. The mono-PEG-IL-10 of claim 2, wherein the linker is a linear C_3 alkyl.

6. The mono-PEG-IL-10 of claim 1, wherein a PEG molecule is covalently attached to the alpha amino group of one N-terminus of IL-10 via a linear C_3 alkyl linker.

7. A PEG-IL-10 comprising the formula: [X--O(CH_{2CH2O})$_n$].s ub.b--L--NH-IL-10, where X is H or C_{1-4} alkyl, n is 20 to 2300, b is 1 to 9 and L is a C_{1-11} alkyl linker moiety which is covalently attached to nitrogen (N) of the alpha amino group at the amino terminus of one IL-10 subunit, provided that when b is greater than 1 the total of n does not exceed 2300.

8. A PEG-IL-10 of claim 7, wherein L is --$CH_{2CH2CH2}$--.

9. A pharmaceutical composition, comprising a mono-PEG-IL-10 of claim 1 in combination with a pharmaceutically acceptable carrier.

10. A method of treating inflammation in an individual in need of such treatment, comprising administering to the individual a therapeutically effective amount of a pharmaceutical composition of claim 9.

11. A process for preparing a mono-PEG-IL-10, comprising the step of: reacting IL-10 with an activated PEG-aldehyde linker in the presence of a reducing agent to form the mono-PEG-IL-10, wherein the linker is covalently attached to one amino acid residue of the IL-10.

12. The process of claim 11 wherein: (a) the reducing agent is sodium cyanoborohydride; (b) the activated PEG-aldehyde linker is PEG-propionaldehyde; (c) the PEG is a methoxy-PEG; (d) the linker is multi-armed; (e) the ratio of IL-10 to the sodium cyanoborohydride is from about 1:0.5 to 1:50; (f) the total molecular mass of all PEG comprising the PEG-aldehyde linker is from 3,000 daltons to 60,000 daltons; or (g) the reacting step is performed at a pH of 5.5 to 7.8.

13. The process of claim 11, wherein the ratio of IL-10 to the sodium cyanoborohydride is 1:5 to 1:15.

14. The process of claim 11, wherein the total molecular mass of all PEG comprising the PEG-aldehyde linker is from 10,000 daltons to 36,000 daltons.

15. The process of claim 11, wherein the reacting step is performed at a pH of 6.3 to 7.5.

16. The process of claim 11, further comprising a step selected from: incubating the mono-PEG-IL-10 product in a buffer at pH 5.0 to 9.0; and treating the mono-PEG-IL-10 product with 0.05 to 0.4 M hydroxylamine HCl salt.

17. A process for preparing a mono-PEG-IL-10, comprising the step of: reacting IL-10 with an activated PEG-propionaldehyde linker in the presence of sodium cyanoborohydride, wherein the molar ratio of IL-10 to sodium cyanoborohydride is from about 1:5 to about 1:15, at a pH of about 6.3 to about 7.5 and a temperature of from 18° C. to 25° C. form the mono-PEG-IL-10, wherein the linker is covalently attached to one amino acid residue of the IL-10.

18. The process of claim 17, wherein the total molecular mass of all PEG comprising the PEG-aldehyde linker is from 10,000 daltons to 36,000 daltons.

19. The process of claim 17, further comprising a step selected from: incubating the mono-PEG-IL-10 product in a TRIS buffer at pH 7.0 to 8.0; and treating the mono-PEG-IL-10 product with 0.05 to 0.4 M hydroxylamine HCl salt.

20. A PEG-IL-10 prepared according to a process of claim 11.

L20 ANSWER 3 OF 5 USPATFULL on STN
Full Text
AN 2001:142470 USPATFULL
TI Methods for conversion of protein isoforms
IN **Cannon-Carlson, Susan**, Wayne, NJ, United States
Frei, Andres, Freehold, NJ, United States
Lee, Seoju, Edison, NJ, United States
Mengisen, Roland, Freehold, NJ, United States
Voloch, Marcio, New York City, NY, United States
Wylie, David C., Cranford, NJ, United States
PA Schering Corporation, Kenilworth, NJ, United States (U.S. corporation)
PI US 6281337 B1 20010828
AI US 1999-441653 19991112 (9)
PRAI US 1998-107978P 19981112 (60)
DT Utility
FS GRANTED
EXNAM Primary Examiner: Kemmerer, Elizabeth; Assistant Examiner: Landsman, Robert S.
LREP Wyatt, Donald W.
CLMN Number of Claims: 17
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 613
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:
1. A method for increasing the yield of an interferon alpha composition, comprising converting a pyruvate adjunct isoform of interferon alpha into interferon alpha by exposing said pyruvate adjunct isoform of interferon alpha to a solution having a pH of at least 5.0.

2. The method of claim 1, wherein said interferon alpha is interferon alpha 2b.

3. The method of claim 2, wherein said adjunct isoform is exposed to acid solution having a pH in the range of about 5.2 to about 5.6.

4. The method of claim 3, wherein said acid solution is at about pH 5.5.

5. The method of claim 4, wherein said acid solution at 34-40° C.

6. The method of claim 5, wherein said acid solution comprises an antioxidant.

7. The method of claim 6, wherein said antioxidant comprises methionine.

8. The method of claim 7, wherein said methionine is at a concentration of 5-40 mM.

9. The method of claim 1, wherein said adjunct isoform is exposed to zinc solution.

10. The method of claim 9, wherein said zinc solution is at about pH 7.8 to about pH 8.6.

11. The method of claim 10, wherein said zinc solution is at 30-38° C.

12. The method of claim 11, wherein said zinc solution comprises an antioxidant.

13. The method of claim 12, wherein said antioxidant comprises methionine.

14. The method of claim 13, wherein said methionine is at a concentration of 5-40 mM.

15. The method of claim 3, wherein said is exposed to said solution for 24-30 hours.

16. The method of claim 11, wherein the ratio of said Zinc to said isoform is 0.6 to 1.0.

17. The method of claim 16, wherein said isoform is exposed to said solution until 80% of said isoform is converted to interferon alpha.

L20 ANSWER 4 OF 5 USPATFULL on STN
Full Text
AN 1998:7167 USPATFULL
TI Purification of bacterially expressed human interleukin-10
IN Vellekamp, Gary, Glen Ridge, NJ, United States
Cannon-Carlson, Susan, Wayne, NJ, United States
Tang, John, Livingston, NJ, United States
PA Schering Corporation, Kenilworth, NJ, United States (U.S. corporation)
PI US 5710251 19980120
WO 9420525 19940915
AI US 1995-495558 19951218 (8)
WO 1994-US1909 19940303
19951218 PCT 371 date
19951218 PCT 102(e) date
RLI Continuation-in-part of Ser. No. US 1993-26942, filed on 5 Mar 1993, now patented, Pat. No. US 5328989
DT Utility
FS Granted
EXNAM Primary Examiner: Guzo, David; Assistant Examiner: Degen, Nancy T.
LREP McLaughlin, Jaye P., Dulak, Norman C.
CLMN Number of Claims: 9
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 835
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:
1. A method for purifying bacterially expressed human Interleukin-10 (IL-10) contained within a solution comprising: (a) applying a solution containing IL-10 to a cation exchange chromatography column thereby obtaining fractions containing IL-10; (b) applying the IL-10-containing fractions from step (a) to an anion exchange chromatography column thereby obtaining fractions containing IL-10; (c) applying the IL-10-containing fractions from step (b) to a hydroxyapatite chromatography chromatography thereby obtaining fractions containing a single isolated dimer of IL-10.

2. The method of claim 1 wherein the cation exchange chromatography column from step (a) is comprised of sulfonate exchange groups attached to a support matrix.

3. The method of claim 2 wherein the support matrix is agarose.

4. The method of claim 1 wherein the anion exchange chromatography column is comprised of quaternary amino ethyl exchange groups attached to a support matrix.

5. The method of claim 4 wherein the support matrix is agarose.

6. The method of claim 1 further comprising applying the IL-10-containing fractions obtained from step (c) of claim 1 to a gel filtration chromatography column to obtain dimeric IL-10 substantially free of high and low molecular weight impurities.

7. The method of claim 6 wherein the material in the gel filtration chromatography column has a fractionation range of from 1 to 600 kDa.

8. The method of claim 1 wherein prior to step (a) the bacterially expressed human IL-10 is extracted from bacteria in inclusion bodies, denatured and refolded into biologically active human IL-10.

9. A method for separating non-acetylated homodimers of Interleukin-10 (IL-10) from acetylated IL-10 homodimers and from acetylated IL-10 heterodimers contained within a solution comprising: applying the solution to an anion exchange chromatography column under conditions in which the non-acetylated homodimers are separated from the acetylated homodimers and from the acetylated heterodimers.

L20 ANSWER 5 OF 5 USPATFULL on STN
Full Text
AN 94:60238 USPATFULL
TI Purification of human interleukin-10 from a cell culture medium
IN Vellekamp, Gary, Glen Ridge, NJ, United States
Cannon-Carlson, Susan, Wayne, NJ, United States

Tang, John, Livingston, NJ, United States
PA Schering-Plough, Kenilworth, NJ, United States (U.S. corporation)
PI US 5328989 19940712
AI US 1993-26942 19930305 (8)
DT Utility
FS Granted
EXNAM Primary Examiner: Russel, Jeffrey E.; Assistant Examiner: Gromet, Nancy J.
LREP Lunn, Paul G., Dulak, Norman C., Kanstad, Steinar V.
CLMN Number of Claims: 17
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 471
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:

1. A method for purifying Interleukin-10 (IL-10) from a cell culture medium comprising: (a) subjecting the culture medium containing IL-10 to cation exchange chromatography thereby obtaining fractions containing IL-10; (b) subjecting the IL-10-containing fractions from step (a) to anion exchange chromatography thereby obtaining fractions containing IL-10; (c) subjecting the IL-10-containing fractions from step (b) to hydroxyapatite chromatography thereby obtaining fractions containing a single isolated dimer of IL-10.

2. The method of claim 1 wherein the cation exchange chromatography from step (a) uses a column comprised of sulfonate exchange groups attached to a support matrix.

3. The method of claim 2 wherein the support matrix is agarose.

4. The method of claim 1 wherein the anion exchange chromatography uses a column comprised of quartenary amino ethyl exchange groups attached to a support matrix.

5. The method of claim 4 wherein the support matrix is agarose.

6. The method of claim 1 further comprising applying the IL-10-containing fractions obtained from step (c) of claim 1 to a gel filtration chromatography column to obtain dimeric IL-10 substantially free of high and low molecular weight impurities.

7. The method of claim 6 wherein the gel has a fractionation range of from 1 to 600 kDa.

8. The method of claim 1 wherein the IL-10 is human IL-10.

9. The method of claim 1 wherein IL-10 is secreted in a cell culture medium from eukaryotic cell.

10. A method for separating different dimers of IL-10 present in an IL-10 containing protein fraction comprising: subjecting the IL-10 containing fraction to hydroxyapatite chromatography under conditions wherein the different IL-10 dimers are separated from each other.

11. The method of claim 10 wherein the IL-10 dimers present in the protein fraction are $\Delta 0{:}\Delta 0$, $\Delta 0{:}\Delta 2$ and $\Delta 2{:}\Delta 2$ IL-10 dimers.

12. The method of claim 11 wherein the IL-10 dimer which is collected is the $\Delta 0{:}\Delta 0$ IL-10 dimer.

13. The method of claim 11 wherein the IL-10 dimer which is collected is the $\Delta 0{:}\Delta 2$ IL-10 dimer.

14. The method of claim 11 wherein the IL-10 dimer which is collected is the $\Delta 2{:}\Delta 2$ IL-10 dimer.

15. The method of claim 10 wherein the hydroxyapatite is sintered hydroxyapatite.

16. A method for separating different dimers of a protein contained within a protein fraction wherein the different dimers have different N-terminal amino acid sequences comprising: subjecting the protein fraction to hydroxyapatite chromatography under conditions wherein the different dimers of the protein are separated from each other.

17. A method for separating variants of a protein contained within a protein fraction wherein the variants of the protein have different

N-terminal amino acid sequences comprising: subjecting the protein fraction to hydroxyapatite chromatography under conditions wherein the variants of the protein are separated from each other.

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=> d his

     (FILE 'HOME' ENTERED AT 18:36:36 ON 22 JUN 2005)

     FILE 'USPATFULL' ENTERED AT 18:36:44 ON 22 JUN 2005
L1          9532 S HYDROXYAPATITE
L2          1599 S L1 AND HYDROXYAPATITE/CLM
L3            69 S L2 AND ADENOVIR?
L4            13 S L3 AND ADENOVIR?/CLM
L5            33 S L3 AND AY<2001
L6            27 S L5 NOT L4
L7             1 S US5496926/PN
L8             1 S L7 AND ADENOVIR?
L9             1 S US5624833/PN
L10            1 S L9 AND ADENOVIR?
               E ERICKSON AMY E/IN
L11            1 S E3
               E SHABRAM PAUL W/IN
L12            5 S E3

     FILE 'WPIDS' ENTERED AT 18:46:16 ON 22 JUN 2005
L13         2918 S HYDROXYAPATITE
L14           14 S L13 AND ADENOVIR?
               E SHABRAM P W/IN
L15            6 S E3
               E ERICKSON A E/IN
L16            1 S E3

     FILE 'USPATFULL' ENTERED AT 18:53:21 ON 22 JUN 2005
               E VELLEKAMP G J/IN
L17            7 S E4 OR E5
L18            3 S L17 AND HYDROXYAPATITE
               E CUTLER COLLETTE/IN
L19            1 S E3
               E CANNON-CARLSON S V/IN
               E CANNON CARLSON S V/IN
L20            5 S E4 OR E5

=> file medline
COST IN U.S. DOLLARS                          SINCE FILE      TOTAL
                                                 ENTRY    SESSION
FULL ESTIMATED COST                              43.76     224.57

FILE 'MEDLINE' ENTERED AT 18:59:27 ON 22 JUN 2005

 FILE LAST UPDATED: 22 JUN 2005 (20050622/UP).  FILE COVERS 1950 TO DATE.

 On December 19, 2004, the 2005 MeSH terms were loaded.

 The MEDLINE reload for 2005 is now available.  For details enter HELP
 RLOAD at an arrow promt (=>).  See also:

    http://www.nlm.nih.gov/mesh/
    http://www.nlm.nih.gov/pubs/techbull/nd04/nd04_mesh.html

 OLDMEDLINE now back to 1950.

 MEDLINE thesauri in the /CN, /CT, and /MN fields incorporate the
 MeSH 2005 vocabulary.

 This file contains CAS Registry Numbers for easy and accurate
 substance identification.

=> s hydroxyapatite
L21         9422 HYDROXYAPATITE

=> s l21 and (vir? or retrovir? or adenovir?)
          663873 VIR?
           34438 RETROVIR?
           31060 ADENOVIR?
L22          230 L21 AND (VIR? OR RETROVIR? OR ADENOVIR?)

=> s l22 and py<2001
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12878767 PY<2001
L23 199 L22 AND PY<2001

=> s l23 and purif?
680266 PURIF?
L24 101 L23 AND PURIF?

=> d l24,cbib,ab,1-25

L24 ANSWER 1 OF 101 MEDLINE on STN
2003033301. PubMed ID: 12539718. A study on biological properties of fimbriae of A. viscosus. III. Adherence activity of fimbriae of A. viscosus. Liu T; Li W; Yue S. (College of Stomatology, West China University of Medical Sciences.) Hua xi kou qiang yi xue za zhi = Huaxi kouqiang yixue zazhi = West China journal of stomatology, **(1999 May)** 17 (2) 169-72. Journal code: 9422648. ISSN: 1000-1182. Pub. country: China. Language: Chinese.
AB OBJECTIVE: To investigate the adherence activity of two types of fimbriae of A. viscosus on tooth surface or with S. sanguis 34. METHODS: The inhibited adherence tests, coaggregation tests and the inhibited coaggregation tests were done. RESULTS: 1. The **purified** type I and type II fimbriae inhibited the adherence of A. viscosus to salivary-treated **hydroxyapatite** (SHA) and the two specific IgG to type I and type II fimbriae blocked the adsorption of strain T14V, strain 5519 and strain 5951 to SHA; 2. Only type II fimbria indirectly mediated the visible agglutination of S. sanguis 34 and only IgG to type II fimbriae inhibited coaggregation of strain T14V and strain 5915 with S. sanguis 34. CONCLUSION: Type I and type II fimbriae have adherence ability, and only type II fimbria has the agglutination activity. Additionally, the methods which were used to prepare fimbriae don't damage the biological activity of fimbriae.

L24 ANSWER 2 OF 101 MEDLINE on STN
2001132208. PubMed ID: 11199265. Scaleable chromatographic **purification** process for recombinant adeno-associated **virus** (rAAV). O'Riordan C R; Lachapelle A L; Vincent K A; Wadsworth S C. (Genzyme Corporation, Framingham, MA 01701-9322, USA.. coriordan@genzyme.com) . journal of gene medicine, **(2000 Nov-Dec)** 2 (6) 444-54. Journal code: 9815764. ISSN: 1099-498X. Pub. country: England: United Kingdom. Language: English.
AB BACKGROUND: Adeno-associated **virus** (AAV) is a human parvovirus currently being developed as a vector for gene therapy applications. Traditionally AAV has been **purified** from cell lysates using CsCl gradients; this approach however is not likely to be useful in large-scale manufacturing. Moreover gradient-**purified** AAV vectors tend to be contaminated with significant levels of cellular and **adenoviral** proteins and nucleic acid. To address the issue of **purification** we have developed a process scale method for the rapid and efficient **purification** of recombinant AAV (rAAV) from crude cellular lysates. METHODS: The preferred method for the **purification** of rAAVbetagal includes treatment of **virally** infected cell lysates with both trypsin and nuclease followed by ion exchange chromatography using ceramic **hydroxyapatite** and DEAE-Sepharose in combination with cellufine sulphate affinity chromatography. RESULTS: **Purification** of rAAV particles from crude cellular lysates co-infected with **adenovirus** was achieved using column chromatography exclusively. Column-**purified** rAAV was shown to be greater than 90% pure, free of any detectable contaminating **adenovirus**, biologically active, and capable of directing efficient gene transfer to the lungs of both cotton rats and mice. CONCLUSIONS: This study demonstrates the feasibility of using column chromatography alone for the isolation of highly **purified** rAAV vector. The methods described here are advancements in procedures to **purify** rAAV and are adaptable for commercial production of clinical-grade rAAV vector.

L24 ANSWER 3 OF 101 MEDLINE on STN
20001063126. PubMed ID: 11090756. **Hydroxyapatite**-coated nylon beads as a new reagent to develop a particle agglutination assay system for detecting Japanese encephalitis **virus**-specific human antibodies. Yamamoto A; Nakayama M; Tashiro M; Ogawa T; Kurane I. (Department of Virology 1, National Institute of Infectious Diseases, 1-23-1 Toyama, Shinjuku-ku, 162-8640, Tokyo, Japan.) Journal of clinical virology : official publication of the Pan American Society for Clinical Virology, **(2000 Dec)** 19 (3) 195-204. Journal code: 9815671. ISSN: 1386-6532. Pub. country: Netherlands. Language: English.
AB BACKGROUND: Detection of Japanese encephalitis **virus** (JEV)-specific antibodies is done today by hemagglutination-inhibition assay (HIA), neutralization assay (NTA) and enzyme-linked immunosorbent assay (ELISA). These conventional assays are often difficult to perform in diagnostic laboratories with insufficient resources. An alternative antibody detection kit, which is simple, preservable and inexpensive, is needed for

extended use in rural areas of Asia. OBJECTIVES: (i) Characterization of a new antigen carrier, **hydroxyapatite**-coated nylon (Ha-Ny) beads, and (ii) evaluation of the JEV antigen-coated Ha-Ny beads as a reagent to detect anti-JEV antibodies in human serum samples. STUDY DESIGN: We examined the Ha-Ny beads for **hydroxyapatite** content, precipitation efficiency and protein adsorption ability. We then developed a particle agglutination assay system using the JEV antigen-coated Ha-Ny beads, and tried out the newly developed assay system with reference serum samples. RESULTS: The beads had the ability to adsorb 0.44 mg of lysozyme per gram. Sedimentation speed was 10.2 cm/30 min in phosphate buffered saline (PBS), pH 7.0. Binding of the JEV antigen on Ha-Ny beads was confirmed by scanning electron microscopy (SEM) and ELISA. Eighteen confirmed-human serum samples were tested by the newly developed particle agglutination assay system. The results were consistent with those from HIA, NTA and ELISA. CONCLUSION: The Ha-Ny beads can be applicable to the development of a new JEV antibody-detection kit, which does not require specific laboratory facilities.

L24 ANSWER 4 OF 101 MEDLINE on STN

2000200244. PubMed ID: 10733882. Optimized production and **purification** of Bacillus anthracis lethal factor. Park S; Leppla S H. (Oral Infection and Immunity Branch, National Institute of Dental and Craniofacial Research, Bethesda, Maryland, 20892, USA.) Protein expression and purification, **(2000 Apr)** 18 (3) 293-302. Journal code: 9101496. ISSN: 1046-5928. Pub. country: United States. Language: English.

AB Bacillus anthracis lethal factor (LF) is a 90-kDa zinc metalloprotease that plays an important role in the **virulence** of the organism. LF has previously been **purified** from Escherichia coli and Bacillus anthracis. The yields and purities of these preparations were inadequate for crystal structure determination. In this study, the genes encoding wild-type LF and a mutated, inactive LF (LF-E687C) were placed in an E. coli-Bacillus shuttle vector so that LF was produced with the protective antigen (PA) signal peptide at its N-terminus. The resulting vectors, pSJ115 and pSJ121, express wild-type and mutated LF fusion proteins, respectively. Expression of the LF genes is under the control of the PA promoter and, during secretion, the PA signal peptide is cleaved to release the 90-kDa LF proteins. The wild-type and mutated LF proteins were **purified** from the culture medium using three chromatographic steps (Phenyl-Sepharose, Q-Sepharose, and **hydroxyapatite**). The **purified** proteins were greater than 95% pure and yields (20-30 mg/L) were higher than those obtained in other expression systems (1-5 mg/L). These proteins have been crystallized and are being used to solve the crystal structure of LF. Their potential use in anthrax vaccines is also discussed. Copyright 2000 Academic Press.

L24 ANSWER 5 OF 101 MEDLINE on STN

2000068856. PubMed ID: 10600124. Properties of p-cresol methylhydroxylase flavoprotein overproduced by Escherichia coli. Engst S; Kuusk V; Efimov I; Cronin C N; McIntire W S. (Molecular Biology Division, Department of Veterans Affairs Medical Center, San Francisco, California 94121, USA.) Biochemistry, **(1999 Dec 14)** 38 (50) 16620-8. Journal code: 0370623. ISSN: 0006-2960. Pub. country: United States. Language: English.

AB The alpha(2)beta(2) flavocytochrome p-cresol methylhydroxylase (PCMH) from Pseudomonas putida is composed of a flavoprotein homodimer (alpha(2) or PchF(2); M(r) = 119 kDa) with a cytochrome monomer (beta, PchC; M(r) = 9.3 kDa) bound to each PchF subunit. Escherichia coli BL21(DE3) has been transformed with a vector for expression of the pchF gene, and PchF is overproduced by this strain as the homodimer. During **purification**, it was recognized that some PchF had FAD bound, while the remainder was FAD-free. However, unlike PchF obtained from PCMH **purified** from P. putida, FAD was bound noncovalently. The FAD was conveniently removed from **purified** E. coli-expressed PchF by **hydroxyapatite** chromatography. Fluorescence quenching titration indicated that the affinity of apo-PchF for FAD was sufficiently high to prevent the determination of the dissociation constant. It was found that p-cresol was **virtually** incapable of reducing PchF with noncovalently bound FAD (PchF(NC)), whereas 4-hydroxybenzyl alcohol, the intermediate product of p-cresol oxidation by PCMH, reduced PchF(NC) fairly quickly. In contrast, p-cresol rapidly reduced PchF with covalently bound FAD (PchF(C)), but, unlike intact PCMH, which consumed 4 electron equiv/mol when titrated with p-cresol (2 electrons from p-cresol and 2 from 4-hydroxybenzyl alcohol), PchF(C) accepted only 2 electron equiv/mol. This is explained by extremely slow release of 4-hydroxybenzyl alcohol from reduced PchF(C). 4-Hydroxybenzyl alcohol rapidly reduced PchF(C), producing 4-hydroxybenzaldehyde. It was demonstrated that p-cresol has a charge-transfer interaction with FAD when bound to oxidized PchF(NC), whereas 4-bromophenol (a substrate analogue) and 4-hydroxybenzaldehyde have charge-transfer interactions with FAD when bound to either PchF(C) or PchF(NC). This is the first example of a "wild-type" flavoprotein, which

normally has covalently bound flavin, to bind flavin noncovalently in a stable, redox-active manner.

L24 ANSWER 6 OF 101 MEDLINE on STN
2000060398. PubMed ID: 10592813. Production and **purification** of Bordetella pertussis toxin. Ju C L; Sheu G C; Cheng Y; Lu C H. (National Institute of Preventive Medicine, Taipei, Taiwan, R.O.C.) Zhonghua min guo wei sheng wu ji mian yi xue za zhi = Chinese journal of microbiology and immunology, **(1997 May)** 30 (2) 72-83. Journal code: 8008067. ISSN: 0253-2662. Pub. country: CHINA (REPUBLIC : 1949-). Language: English.
AB Pertussis toxin (PT) is the major protective antigen of acellular pertussis vaccine (aP). We have established an optimal culture condition for the growth of B. pertussis and the production of PT in a laboratory scale fermentor. It was found that when the dissolved oxygen in medium was supplied with pure oxygen instead of air, the yield of PT was dramatically increased (i.e. from 2-3 mg/l using air to 8-10 mg/l using pure oxygen). PT was **purified** by affinity chromatography using **hydroxyapatite** and fetuin-sepharose columns. SDS-PAGE analysis and CHO cell clustering test showed that the **purified** PT was comparable to the reference PT in purity and biological activity. The **purified** PT could be detoxified by formaldehyde (d-PT). The results of CHO cell clustering neutralization assay and ELISA showed that the antibody induced by d-PT in mice was comparable to that induced by PT contained in a commercial DTaP. These results indicated that the immunogenicity of our d-PT was retained after the **purification** and detoxification procedures.

L24 ANSWER 7 OF 101 MEDLINE on STN
1999290073. PubMed ID: 10361722. Generation of a new protein **purification** matrix by loading ceramic **hydroxyapatite** with metal ions--demonstration with poly-histidine tagged green fluorescent protein. Nordstrom T; Senkas A; Eriksson S; Pontynen N; Nordstrom E; Lindqvist C. (Department of Biochemistry and Pharmacy, Abo Akademi University, Finland.) Journal of biotechnology, **(1999 Apr 15)** 69 (2-3) 125-33. Journal code: 8411927. ISSN: 0168-1656. Pub. country: Netherlands. Language: English.
AB The gene encoding the green fluorescent protein (GFP) from the jellyfish Aequorea victoria, was inserted under transcriptional control of the polyhedrin promoter of the Autographa californica nuclear polyhedrosis **virus** and expressed in the Spodoptera frugiperda insect cell line Sf9 during **viral** infection. The baculovirus transfervector pBlueBacHisB was used for constructing the recombinant baculovirus, so that the green fluorescent protein could be tagged with a poly-histidine tail. This fusion protein was utilized as a marker for evaluating the properties of metal ion loaded ceramic **hydroxyapatite** as a matrix in protein **purification**. Ceramic **hydroxyapatite** loaded with Zn(II) was the best choice for **purifying** this poly-histidine tagged GFP, followed by Fe(III) of the metal ions tested. Ni(II) that is superior especially in many poly-histidine **purification** systems did not, when loaded to **hydroxyapatite**, have binding properties comparable to Zn(II) or Fe(III). Elution of poly-histidine tagged GFP was best performed with phosphate buffers or EDTA that could compete with the phosphate molecules in **hydroxyapatite** or complexly bind the metal ions, respectively.

L24 ANSWER 8 OF 101 MEDLINE on STN
1999221316. PubMed ID: 10206475. Kinetics of the reconstituted tricarboxylate carrier from eel liver mitochondria. Zara V; Palmieri L; Franco M R; Perrone M; Gnoni G V; Palmieri F. (Dipartimento di Biologia, Universita di Lecce, Italy.) Journal of bioenergetics and biomembranes, **(1998 Dec)** 30 (6) 555-63. Journal code: 7701859. ISSN: 0145-479X. Pub. country: United States. Language: English.
AB The tricarboxylate carrier from eel liver mitochondria was **purified** by chromatography on **hydroxyapatite** and Matrix Gel Blue B and reconstituted into liposomes by removal of the detergent with Amberlite. Optimal transport activity was obtained by using a phospholipid concentration of 11.5 mg/ml, a Triton X- 114/phospholipid ratio of 0.9, and ten passages through the same Amberlite column. The activity of the carrier was influenced by the phospholipid composition of the liposomes, being increased by cardiolipin and phosphatidylethanolamine and decreased by phosphatidylinositol. The reconstituted tricarboxylate carrier catalyzed a first-order reaction of citrate/citrate or citrate/malate exchange. The maximum transport rate of external [14C]citrate was 9.0 mmol/min per g of tricarboxylate carrier protein at 25 degrees C and this value was **virtually** independent of the type of substrate present in the external or internal space of the liposomes. The half-saturation constant (Km) was 62 microM for citrate and 541 microM for malate. The activation energy of the citrate/citrate exchange reaction was 74 kJ/mol from 5 to 19 degrees C and 31 kJ/mol from 19 to 35 degrees C. The rate of the exchange had an external pH optimum of 8.

L24 ANSWER 9 OF 101 MEDLINE on STN

1999128112. PubMed ID: 9930922. **Purified** soluble guanylyl cyclase expressed in a baculovirus/Sf9 system: stimulation by YC-1, nitric oxide, and carbon monoxide. Hoenicka M; Becker E M; Apeler H; Sirichoke T; Schroder H; Gerzer R; Stasch J P. (DLR Institute of Aerospace Medicine, Cologne, Germany.. hoenicka@pbmail.me.kp.dlr.de) . Journal of molecular medicine (Berlin, Germany), **(1999 Jan)** 77 (1) 14-23. Journal code: 9504370. ISSN: 0946-2716. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB Soluble guanylyl cyclase (sGC) is the main receptor for nitric oxide, a messenger molecule with multiple clinical implications. Understanding the activation of sGC is an important step for establishing new therapeutic principles. We have now overexpressed sGC in a baculovirus/Sf9 system optimized for high protein yields to facilitate spectral and kinetic studies of the activation mechanisms of this enzyme. It was expressed in a batch fermenter using a defined mixture of **viruses** encoding the alpha and beta1 subunits of the rat lung enzyme. The expressed enzyme was **purified** from the cytosolic fraction by anion exchange chromatography, **hydroxyapatite** chromatography, and size exclusion chromatography. By use of this new method 2.5 l culture yielded about 1 mg of apparently homogeneous sGC with a content of about one heme per heterodimer without the need of a heme reconstitution step. The enzyme did not contain stoichiometric amounts of copper. The basal activities of the **purified** enzyme were 153 and 1259 nmol min(-1) mg(-1) in the presence of Mg2+ and Mn2+, respectively. The nitric oxide releasing agent 2-(N,N-diethylamino)-diazenolate-2-oxide (DEA/NO) stimulated the enzyme 160-fold with Mg2+, whereas the NO-independent activator 3-(5'-hydroxymethyl-2'-furyl)-1-benzylindazole (YC-1) induced an increase in the activity of 101-fold at a concentration of 300 microM. The combination of DEA/NO (10 microM) and YC-1 (100 microM) elicited a dose-dependent synergistic stimulation with a maximum of a 792-fold increase over the basal activity in the presence of Mg2+, resulting in a specific activity of 121 micromol min(-1) mg(-1). The synergistic stimulation of DEA/NO and YC-1 was attenuated by the sGC inhibitor 1H-(1,2,4)oxadiazole(4,3-a)quinoxalin-1-one (ODQ) (10 microM) by 94%. In a different experimental setup a saturated carbon monoxide solution in the absence of ambient oxygen or NO stimulated the enzyme 15-fold in the absence and 1260-fold in the presence of YC-1 compared to an argon control. The heme spectra of the enzyme showed a shift of the Soret peak from 432 to 399 and 424 nm in the presence of DEA/NO or carbon monoxide, respectively. The heme spectra were not affected by YC-1 in the absence or in the presence of DEA/NO or of carbon monoxide, which reflects the fact that YC-1 does not interact directly with the heme group of the enzyme. In summary, this study shows that our expression/**purification** procedure is suitable for producing large amounts of highly pure sGC which contains one heme per heterodimer without a reconstitution step. The activator experiments show that in a synergistic stimulation with YC-1 sGC can be activated maximally both by nitric oxide and by carbon monoxide and that YC-1 does not directly act via heme. The described method should help to facilitate the investigation of the new therapeutic principle of NO-independent guanylyl cyclase activators.

L24 ANSWER 10 OF 101 MEDLINE on STN

1999055198. PubMed ID: 9836747. Isolation of an RNA-directed RNA polymerase-specific cDNA clone from tomato. Schiebel W; Pelissier T; Riedel L; Thalmeir S; Schiebel R; Kempe D; Lottspeich F; Sanger H L; Wassenegger M. (Max-Planck-Institut fur Biochemie, Abteilung Viroidforschung, D-82152 Martinsried, Germany.) Plant cell, **(1998 Dec)** 10 (12) 2087-101. Journal code: 9208688. ISSN: 1040-4651. Pub. country: United States. Language: English.

AB A 3600-bp RNA-directed RNA polymerase (RdRP)-specific cDNA comprising an open reading frame (ORF) of 1114 amino acids was isolated from tomato. The putative protein encoded by this ORF does not share homology with any characterized proteins. Antibodies that were raised against synthetic peptides whose sequences have been deduced from the ORF were shown to specifically detect the 127-kD tomato RdRP protein. The immunoresponse to the antibodies correlated with the enzymatic activity profile of the RdRP after chromatography on Q-, poly(A)-, and poly(U)-Sepharose, **hydroxyapatite**, and Sephadex G-200 columns. DNA gel blot analysis revealed a single copy of the RdRP gene in tomato. RdRP homologs from petunia, Arabidopsis, tobacco, and wheat were identified by using polymerase chain reaction. A sequence comparison indicated that sequences homologous to RdRP are also present in the yeast Schizosaccharomyces pombe and in the nematode Caenorhabditis elegans. The previously described induction of RdRP activity upon **viroid** infection is shown to be correlated with an increased steady state level of the corresponding mRNA. The possible involvement of this heretofore functionally elusive plant RNA polymerase in homology-dependent gene silencing is discussed.

L24 ANSWER 11 OF 101 MEDLINE on STN

1998380387. PubMed ID: 9712790. Isolation and characterization of two proteins from Moraxella catarrhalis that bear a common epitope. McMichael J C; Fiske M J; Fredenburg R A; Chakravarti D N; VanDerMeid K R; Barniak V; Caplan J; Bortell E; Baker S; Arumugham R; Chen D. (Wyeth-Lederle Vaccines and Pediatrics, West Henrietta, New York 14586-9728, USA.. John_McMichael@internetmail.pr.cyanamid.com) . Infection and immunity, **(1998 Sep)** 66 (9) 4374-81. Journal code: 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.

AB The UspA1 and UspA2 proteins of Moraxella catarrhalis are potential vaccine candidates for preventing disease caused by this organism. We have characterized both proteins and evaluated their vaccine potential using both in vitro and in vivo assays. Both proteins were **purified** from the O35E isolate by Triton X-100 extraction, followed by ion-exchange and **hydroxyapatite** chromatography. Analysis of the sequences of internal peptides, prepared by enzymatic and chemical cleavage of the proteins, revealed that UspA1 and UspA2 exhibited distinct structural differences but shared a common sequence including an epitope recognized by the monoclonal antibody 17C7. By sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), **purified** UspA1 exhibited a molecular weight of approximately 350, 000 when unheated and a molecular weight of 100,000 after being heated for 10 min at 100 degreesC. In contrast, **purified** UspA2 exhibited an apparent molecular weight of 240,000 by SDS-PAGE that did not change with the length of time of heating. Their sizes as determined by gel filtration were 1,150,000 and 830,000 for UspA1 and UspA2, respectively. Preliminary results indicate the proteins have separate functions in bacterial pathogenesis. **Purified** UspA1 was found to bind HEp-2 cells, and sera against UspA1, but not against UspA2, blocked binding of the O35E isolate to the HEp-2 cells. UspA1 also bound fibronectin and appears to have a role in bacterial attachment. **Purified** UspA2, however, did not bind fibronectin but had an affinity for vitronectin. Both proteins elicited bactericidal antibodies in mice to homologous and heterologous disease isolates. Finally, mice immunized with each of the proteins, followed by pulmonary challenge with either the homologous or a heterologous isolate, cleared the bacteria more rapidly than mock-immunized mice. These results suggest that UspA1 and UspA2 serve different **virulence** functions and that both are promising vaccine candidates.

L24 ANSWER 12 OF 101 MEDLINE on STN

1998163279. PubMed ID: 9504751. Identification of hepatitis E **virus** in clinical specimens: amplification of **hydroxyapatite-purified virus** RNA and restriction endonuclease analysis. Gouvea V; Cohen S J; Santos N; Myint K S; Hoke C Jr; Innis B L. (Department of Virus Diseases, Walter Reed Army Institute of Research, Washington, DC 20307-5100, USA.. dr._vera_gouvea@wrsmtp-ccmail.army.mil) . Journal of virological methods, **(1997 Dec)** 69 (1-2) 53-61. Journal code: 8005839. ISSN: 0166-0934. Pub. country: Netherlands. Language: English.

AB A multi-site nested reverse transcription and polymerase chain reaction (RT-PCR) followed by restriction endonuclease analysis (REA) was developed to identify hepatitis E **virus** (HEV) in clinical specimens. Four sets of primers were selected to amplify regions in the HEV genome supposed to encode the helicase, polymerase, and parts of the **viral** capsid protein. Digestion of the nested PCR products with HinfI, HaeII, AvaII, BglI, KpnI, SmaI, or EcoRI generated readily recognizable profiles that confirm the HEV sequences and/or distinguish the unique Mexico genotype (our positive control) from all other isolates (Asian genotype). In addition, the **hydroxyapatite** (HA) adsorption method was compared to other adsorption and extraction methods widely used to **purify viral** RNA from clinical specimens for RT-PCR. All methods presented the same sensitivity of recovery of HEV RNA, but only the adsorption methods efficiently removed fecal enzymatic inhibitors. The HA method gave the best results and was the most economic in terms of time, cost, manipulations and reagents. The method was validated by screening a small number of serum and fecal specimens available from patients with acute non-A,B,C hepatitis in Nepal. HEV RNA was identified in half (5/11) of the fecal specimens obtained from patients with evidence of recent HEV infection, but in none of the 14 patients without a serological marker for hepatitis E.

L24 ANSWER 13 OF 101 MEDLINE on STN

97445050. PubMed ID: 9300039. **Purification** and characterization of the protein kinase encoded by the UL13 gene of herpes simplex **virus** type 2. Daikoku T; Shibata S; Goshima F; Oshima S; Tsurumi T; Yamada H; Yamashita Y; Nishiyama Y. (Laboratory of Virology, Nagoya University School of Medicine, Japan.. daikokut@tsuru.med.nagoya-u.ac.jp) . Virology, **(1997 Aug 18)** 235 (1) 82-93. Journal code: 0110674. ISSN: 0042-6822. Pub. country: United States. Language: English.

AB The proteins encoded by the UL13 genes of herpes simplex **virus** types 1 (HSV-1) and 2 (HSV-2) have been predicted to be protein kinases. To identify the UL13 gene product, we have raised a rabbit polyclonal

antiserum against a His.Tag-HSV-1 UL13 fusion protein. The antibody specifically reacted with the 60-kDa UL13 fusion protein expressed in Escherichia coli and also recognized 56- to 57-kDa late proteins in nuclear fractions of HSV-1- and HSV-2-infected cells. On the other hand, novel casein kinase activity was induced at the late stage of infection when Vero cells were infected with HSV-1 and HSV-2. The induction of the activity was most prominent in the nuclear fractions of HSV-2-infected cells and therefore we **purified** the protein kinase (PK) from the nuclear extracts by successive column chromatography (phosphocellulose, DEAE-cellulose, and **hydroxyapatite**) using casein as an exogenous substrate. The final preparation of the enzyme contained a single major protein with an apparent molecular weight of 56 kDa which was specifically reacted with the UL13 antiserum. The PK activity was optimal in the absence of NaCl and at relatively high pH. Acidic proteins such as casein and phosvitin were efficiently phosphorylated by the PK. A basic protein, protamine, which is the best substrate for the HSV-2 US3 PK, was not detectably phosphorylated but histone was a relatively good substrate for the UL13 PK. Phosphoamino acid analysis revealed that the PK phosphorylated serine and threonine but not tyrosine. Moreover the enzyme was found to be highly resistant to heparin, a potent inhibitor of casein kinase II (CK II) and also resistant to CK I-7, a synthetic inhibitor of CK I, but very sensitive to a bioflavonoid quercetin. These results indicate that the HSV-2 UL13 PK had unique catalytic properties different from those of cellular CK I, CK II, and the **viral** PK encoded by the US3 gene. We have also determined the complete nucleotide sequence of the HSV-2 UL13 gene. The overall amino acid homology between the HSV-2 and HSV-1 UL13 PKs was 85.9% and the homology was highly conserved in the C-terminal region.

L24 ANSWER 14 OF 101 MEDLINE on STN
97433272. PubMed ID: 9288912. Expression of natural and synthetic genes encoding herpes simplex **virus** 1 protease in Escherichia coli and **purification** of the protein. Apeler H; Gottschalk U; Guntermann D; Hansen J; Massen J; Schmidt E; Schneider K H; Schneidereit M; Rubsamen-Waigmann H. (Pharma Research, Bayer AG, Wuppertal, Germany.) European journal of biochemistry / FEBS, **(1997 Aug 1)** 247 (3) 890-5. Journal code: 0107600. ISSN: 0014-2956. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.
AB An attractive target for anti-herpes chemotherapy is the herpes simplex **virus** 1 (HSV-1) protease encoded by the UL26 gene. Studies with HSV-1 strains that harbour mutations in the protease gene have demonstrated that the protease is essential for DNA packaging and **virus** maturation. The UL26 translation product is 635 amino acids long and undergoes autoproteolytic processing between residues Ala247/Ser248 and Ala610/Ser611. The N-terminal processing product (amino acids 1-247) contains the protease domain. To perform crystallization studies and high throughput screening for potent inhibitors, large amounts of the HSV-1 protease are required. However, expression of the natural HSV-1 protease gene in Escherichia coli using a T7-promoter-regulated system is low and does not allow for the efficient production of larger amounts of highly **purified** enzyme. In this report, we describe the use of a synthetic protease gene with optimized E. coli codon usage. The level of protease expression was at least 20 times higher with the synthetic gene as compared to the natural UL26 gene. The HSV-1 protease was **purified** to homogeneity in three steps using mixed-bed ion-exchange chromatography, affinity chromatography, and **hydroxyapatite** chromatography.

L24 ANSWER 15 OF 101 MEDLINE on STN
97420842. PubMed ID: 9275272. Site-specific endonuclease AbaI from Azospirillum brasilense UQ 1796 is an isoschizomer of endonuclease BclI. Zabaznaya E V; Nikiforov V V; Zheleznaya L A; Matvienko N I. (Institute of Biochemistry and Physiology of Plants and Microorganisms, Russian Academy of Sciences, Saratov, Russia.. root@ibppm.saratov.su) . Biochemistry. Biokhimiia, **(1997 Apr)** 62 (4) 343-9. Journal code: 0376536. ISSN: 0006-2979. Pub. country: RUSSIA: Russian Federation. Language: English.
AB The site-specific endonuclease AbaI was isolated and **purified** to functional purity from the soil nitrogen-fixing bacterium Azospirillum brasilense UQ 1796. **Purification** included successive chromatography on columns with phosphocellulose, heparin-Sepharose, and **hydroxyapatite**. The **purified** enzyme recognizes the palindromic DNA sequence 5'-T decreases ATCA-3' and cleaves it as shown by the arrow. The isolated enzyme belongs to class II restriction endonuclease and is an isoschizomer of endonuclease BclI. The enzyme of AbaI is active at 26-56 degrees C. The optimal temperature is 48 degrees C and the optimal buffer is LRB.

L24 ANSWER 16 OF 101 MEDLINE on STN
97327744. PubMed ID: 9210421. **Purification** and properties of alpha-mannosidase II from Golgi-like membranes of baculovirus-infected Spodoptera frugiperda (IPLB-SF-21AE) cells. Ren J; Castellino F J;

Bretthauer R K. (Department of Chemistry and Biochemistry and the Center for Transgene Research, University of Notre Dame, Notre Dame, IN 46556, USA.) Biochemical journal, **(1997 Jun 15)** 324 (Pt 3) 951-6. Journal code: 2984726R. ISSN: 0264-6021. Pub. country: ENGLAND: United Kingdom. Language: English.

AB An alpha-mannosidase II-like activity was identified in baculovirus-infected Spodoptera frugiperda (IPLB-SF21-AE) cells. The enzyme responsible was **purified** from Golgi-type membranes to apparent homogeneity by using a combination of steps including DEAE-cellulose, **hydroxyapatite**, concanavalin A-Sepharose and gel filtration chromatography. The molecular mass of this **purified** protein was approx. 120 kDa by SDS/PAGE under reducing conditions and approx. 240 kDa under non-reducing conditions, indicating that the enzyme is a disulphide-linked dimer. Substrates demonstrated to undergo hydrolysis with this enzyme were GlcNAc-Man5-GlcNAc-GlcNAc (non-reduced and reduced) and p-nitrophenyl alpha-d-mannopyranoside. The oligosaccharide substrate was converted into GlcNAc-Man3-GlcNAc-GlcNAc through an intermediate GlcNAc-Man4-GlcNAc-GlcNAc. Treatment of the isolated intermediate oligosaccharide with endoglycosidase H resulted in its conversion into GlcNAc-Man4-GlcNAc. This indicated that it contained the alpha-1,3-linked mannose residue on the alpha-1,6-linked mannose arm and showed that the alpha-1,6-linked mannose residue on the alpha-1,6-linked mannose arm had been preferentially hydrolysed by the mannosidase. The oligosaccharide lacking the beta-1,2-linked GlcNAc residue on the alpha-1,3-linked mannose arm (Man5-GlcNAc-GlcNAc) was not hydrolysed in the presence of the enzyme. Metal ions were not required for enzymic activity on any of the substrates, but Cu2+ was strongly inhibitory. The activity of the enzyme was inhibited at low concentrations of swainsonine, but much higher concentrations of 1-deoxymannojirimycin were required to achieve inhibition. All of these properties are characteristic of mannosidase II enzymes from other eukaryotic tissues. The presence of mannosidase II in lepidopteran insect cells would allow entry of N-linked glycoproteins into the complex processing reaction pathway or into the terminal Man3-GlcNAc-GlcNAc pathway.

L24 ANSWER 17 OF 101 MEDLINE on STN

97163301. PubMed ID: 9009069. NAD-independent lactate and butyryl-CoA dehydrogenases of Clostridium acetobutylicum P262. Diez-Gonzalez F; Russell J B; Hunter J B. (Section of Microbiology, Wing Hall, Cornell University, Ithaca, NY 14853, USA.) Current microbiology, **(1997 Mar)** 34 (3) 162-6. Journal code: 7808448. ISSN: 0343-8651. Pub. country: United States. Language: English.

AB Clostridium acetobutylicum P262 cells that were growing on lactate and acetate had an NAD-independent lactate dehydrogenase(iLDH) activity of 200 nmol mg protein-1 min-1.Ammonium sulfate precipitation and DEAE cellulose caused a 35-fold **purification**. Gel filtration indicated that the iLDH had a molecular weight of approximately 55 kDa, but two bands were always observed. Phenyl sepharose could not separate the two proteins, and **hydroxyapatite** caused a complete loss of activity. The semi-**purified** iLDH had a Vmax of 13,000 nmol mg protein-1 min-1 and a Km value of 3.5 mM for D-lactate. The Vmax and Km values for L-lactate were 300 nmol mg protein-1 min-1 and 0.7 mM. The iLDH had a pH optimum of 7.5, was not activated by fructose-1,6-bisphosphate (FDP), and could be coupled to either 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) or dichlorophenol-indophenol (DCPIP), but not methyl viologen (MV) or benzyl viologen (BV). The iLDH did not have strong absorbance between 500 and 300 nm, and trichloroacetic acid or acid ammonium sulfate extracts had **virtually** no fluorescence at 450 nm. The crude extracts also had MTT-linked butyryl-CoA dehydrogenase activity (60 nmol mg protein-1 min-1). The NAD-independent butyryl-CoA dehydrogenase eluted from DEAE-cellulose as two fractions. The yellow fraction was extremely unstable, but the green fraction could be stored for short periods of time at 5 degrees C. The green-colored butyryl-CoA dehydrogenase had strong absorption at 450 nm, and gel filtration indicated that it had a molecular weight of 90 kDa. The NAD-independent butyryl-CoA dehydrogenase could be coupled to MTT, DCPIP, or MV, but not BV. Because the NAD-independent lactate and butyryl-CoA dehydrogenase could both be linked to low potential carriers, these two enzymes may function as oxidation-reduction system in vivo.

L24 ANSWER 18 OF 101 MEDLINE on STN

97067132. PubMed ID: 8910534. Efficient **purification** and reconstitution of P-glycoprotein for functional and structural studies. Dong M; Penin F; Baggetto L G. (Insitut de Biologie et Chimie des Proteines, UPR 412 CNRS, 7 Passage du Vercors, F-69367 Lyon Cedex 07, France.. lgb@ibcp.fr) . Journal of biological chemistry, **(1996 Nov 15)** 271 (46) 28875-83. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Plasma membrane P-glycoprotein is known as an ATP-dependent drug efflux

pump that confers multidrug resistance to tumor cells. None of the reported **purification** procedures worked properly for our P-glycoprotein-overproducing cell lines, i.e. murine lymphoid leukemia P388/ADR25, rat hepatoma AS30-D/COL10, and human lymphoblastic leukemia CEM/VLB5 cells. We have thus developed a general procedure for efficient **purification** of P-glycoprotein by combining solubilization with sodium dodecyl sulfate and chromatography on ceramic **hydroxyapatite**. This procedure was successful for the three cell lines and yielded 70% of the P-glycoprotein present in the starting plasma membranes with more than 99% purity. After exchanging sodium dodecyl sulfate into dodecyl maltoside and reconstitution into liposomes, **purified** P-glycoprotein exhibited a specific ATPase activity of about 200 nmol/min/mg, which was very similar to that obtained for P-glycoprotein solubilized and **purified** with 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonic acid. This ATPase activity was sensitive to orthovanadate inhibition and stimulated by verapamil and other drugs. More importantly, drug transport properties of the reconstituted P-glycoprotein were comparable with those of P-glycoprotein embedded in plasma membranes. Since it is **virtually** devoid of lipids, this preparation is suitable for both functional and structural investigations.

L24 ANSWER 19 OF 101 MEDLINE on STN

96353512. PubMed ID: 8737719. The reactivity of sera from patients with systemic lupus erythematosus to seven different species of single and double stranded deoxyribonucleic acids. Yu C L; Huang M H; Tsai C Y; Sun K H; Hsieh S C; Tsai Y Y; Tsai S T; Yu H S; Han S H. (Department of Medicine, Veterans General Hospital-Taipei, Taiwan, ROC.) Clinical and experimental rheumatology, **(1996 Mar-Apr)** 14 (2) 137-44. Journal code: 8308521. ISSN: 0392-856X. Pub. country: Italy. Language: English.

AB OBJECTIVE: Anti-DNA antibodies are frequently found in the serum of patients with systemic lupus erythematosus (SLE). To understand whether the avidity of SLE sera to different species of single-stranded (ss) and double-stranded (ds) DNA is different or not, the reactivity of active SLE sera to seven species of DNA from **viral**, bacterial, piscine, and mammalian sources was compared. METHODS: Nineteen sera from patients with active SLE were studied for their reactivity to different ssDNA and dsDNA from Escherichia coli (EC), Micrococcus lysodeikticus (ML), Clostridium perfringens (CP), calf thymus (CT), salmon testis (ST), human placenta (HP) and lambda phage by ELISA. The dsDNA was **purified** by treating it with S1 nuclease and proteinase K, followed by Sephacryl S-300 gel filtration. The ssDNA was **purified** by absorption on a **hydroxyapatite** column after heat-cleavage of the dsDNA. RESULTS: The reactivity of SLE sera to 7 species of dsDNA was not significantly different and they recognized a more widely shared epitope. In contrast, the reactivity of these sera to 7 species of ssDNA was erratic and the antigens could be grouped into high (CP and HP), medium (EC, ML, CT, and ST) and low (lambda-phage) antigenicities. CONCLUSION: The anti-ssDNA and anti-dsDNA antibodies of SLE patients recognize more widely shared determinants on the DNA of seven different species. Lambda-phage DNA shows the poorest immunogenicity among them.

L24 ANSWER 20 OF 101 MEDLINE on STN

96110914. PubMed ID: 8557373. Immunosuppressive factor from Actinobacillus actinomycetemcomitans down regulates cytokine production. Kurita-Ochiai T; Ochiai K. (Department of Microbiology, Nihon University School of Dentistry at Matsudo, Chiba, Japan.) Infection and immunity, **(1996 Jan)** 64 (1) 50-4. Journal code: 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.

AB A cytoplasmic soluble fraction of Actinobacillus actinomycetemcomitans Y4 was isolated and characterized as suppressing mitogen-stimulated proliferation of and cytokine production by C3H/HeN mouse splenic T cells. This factor, designated suppressive factor 1 (SF1), was isolated from the supernatant of sonicated whole bacteria and **purified** by Q-Sepharose Fast Flow column chromatography, DEAE-Sepharose Fast Flow column chromatography, **hydroxyapatite** high-pressure liquid chromatography (HPLC), and Protein Pack 300 & 125 gel filtration HPLC. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis revealed that the **purified** SF1 migrated as a single band corresponding to a molecular mass of 14 kDa. This molecule was protease labile, heat resistant, and noncytotoxic. N'-terminal sequence analysis revealed no homology with any known peptides of periodontopathic bacteria or with any host-derived growth factors. **Purified** SF1 suppressed the proliferation of mouse splenic T cells which had been stimulated with concanavalin A, as well as suppressing the production of interleukin-2 (IL-2), gamma interferon, IL-4, and IL-5 from CD4+ T cells as 0.1 microgram/ml or more. These data suggest that SF1 produced by the periodontal pathogen A. actinomycetemcomitans functions as a **virulence** factor by down regulating T-cell proliferation and cytokine production at local defense sites.

L24 ANSWER 21 OF 101 MEDLINE on STN
96080290. PubMed ID: 7503803. Cytochrome P450 2A1, 2E1, and 2C9 cDNA-expression by insect cells and partial **purification** using hydrophobic chromatography. Grogan J; Shou M; Andrusiak E A; Tamura S; Buters J T; Gonzalez F J; Korzekwa K R. (Laboratory of Molecular Carcinogenesis, National Cancer Institute, Bethesda, MD 20892, USA.) Biochemical pharmacology, **(1995 Oct 26)** 50 (9) 1509-15. Journal code: 0101032. ISSN: 0006-2952. Pub. country: ENGLAND: United Kingdom. Language: English.

AB High-level expression of three cloned cytochrome P450 enzymes was accomplished using the baculovirus-insect cell expression system. The amount of enzyme expression was enhanced by cell infections in the presence of medium-supplements containing hemin and by growth in suspension cultures. Human cytochromes P450 2E1 and 2C9 and rat cytochrome P450 2A1 were partially **purified** from cell extracts using hydrophobic interaction and **hydroxyapatite** chromatography. The resulting enzymes were of estimated molecular masses similar to those reported previously and analyzed by PAGE. Reconstitution of enzyme activity resulted when the enzymes were incubated together with NADPH-cytochrome P450 reductase, phospholipid, NADPH, and appropriate substrates. The cytochrome P450 activity of the partially **purified** enzymes was comparable to that of the corresponding enzymes expressed in the vaccinia **virus**-Hep G2 system. These results provide evidence for a general means of obtaining cytochrome P450 enzymes for mechanistic, immunochemical, and biophysical investigations.

L24 ANSWER 22 OF 101 MEDLINE on STN
95094156. PubMed ID: 8001029. Proliferation of hematopoietic cell lines induced by a soluble factor derived from human squamous cell carcinomas of the head and neck. Yasumura S; Amoscato A; Hirabayashi H; Lin W C; Whiteside T L. (Department of Pathology, University of Pittsburgh School of Medicine, PA.) Cancer immunology, immunotherapy : CII, **(1994 Dec)** 39 (6) 407-15. Journal code: 8605732. ISSN: 0340-7004. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB The supernatant of a cell line of squamous cell carcinoma of the head and neck (SCCHN), PCI-50, was previously shown to induce activation, promote proliferation and increase antitumor cytotoxicity of freshly **purified** human natural killer (NK) cells and CD4+ T lymphocytes [Arch Otolaryngol Head Neck Surg (1994) in press]. This supernatant was found also to promote the growth of a variety of hematopoietic cell lines, including Jurkat, THP-1, K562, NK-92 or Epstein-Barr-**virus**-transformed B cell lines. The Jurkat cell line was selected as a reporter cell in an 18-h proliferation assay established to measure the growth-promoting activity of PCI-50 supernatant. The presence of soluble tumor-derived factors able to induce proliferation of Jurkat cells was demonstrated in the supernatant produced by several other SCCHN cell lines but not in that produced by a gastric cancer cell line (HR) or renal cell carcinoma line (5117G8). The growth-promoting PCI-50 supernatant was shown to contain 28 +/- 0.5 pg/ml interleukin-6 (IL-6) in vitro but was negative for interferon gamma, IL-1, IL-2, IL-4, tumor necrosis factor alpha, granulocyte/macrophage-colony-stimulating factor and IL-12. The addition of any of these recombinant cytokines to Jurkat cell cultures did not significantly promote growth, while PCI-50 supernatant was consistently growth-stimulatory. This supernatant neither enhanced intracellular Ca2+ concentration in Jurkat cells nor induced up-regulation of activation antigens on the cell surface, although it supported growth of Jurkat cells in the absence of IL-2. The growth-promoting activity in the PCI-50 supernatant was acid-labile at pH 2 for 4 h, heat-resistant at 96 degrees C for 1 h and sensitive to treatments with trypsin and pepsin. Preincubation of the PCI-50 producer cells with tunicamycin or cyclohexamide reduced the level of growth-promoting activity in the supernatant. A partial **purification** of this activity was achieved using Amicon filtration, chromatography on concanavalin-A-Sepharose and then a **hydroxyapatite** column and high-pressure liquid chromatography gel filtration. The partially **purified** glycoprotein had a molecular mass of 50-70 kDa, as determined by gel filtration.

L24 ANSWER 23 OF 101 MEDLINE on STN
95081237. PubMed ID: 7989435. Identification of Norwalk **virus** in artificially seeded shellfish and selected foods. Gouvea V; Santos N; Timenetsky M do C; Estes M K. (Division of Molecular Biological Research and Evaluation, Food and Drug Administration, Washington, DC 20204.) Journal of virological methods, **(1994 Jul)** 48 (2-3) 177-87. Journal code: 8005839. ISSN: 0166-0934. Pub. country: Netherlands. Language: English.

AB A rotavirus dsRNA **purification** protocol was adapted to extract Norwalk ssRNA from artificially contaminated shellfish, and a sensitive reverse transcription-polymerase chain reaction assay for Norwalk **virus** was devised to identify an estimated 20-200 genomic copies. The technique

includes deproteinization with guanidinium isothiocyanate, adsorption of RNA to **hydroxyapatite**, and sequential precipitation with cetyltrimethylammonium bromide and ethanol. The protocol allows high recovery of **viral** RNA free of enzymatic inhibitors from oysters, clams, and a variety of food matrices. Norwalk **virus** sequences were copied and amplified by using primers selected from the polymerase gene. Digestion of the amplified products with restriction enzymes ensured the specificity of the test. This rapid and sensitive assay may significantly improve the prospect for the routine screening of the uncultivatable Norwalk **virus** in food stuffs.

L24 ANSWER 24 OF 101 MEDLINE on STN
94230647. PubMed ID: 8175943. Improved method for **purification** of **viral** RNA from fecal specimens for rotavirus detection. Santos N; Gouvea V. (Division of Molecular Biological Research and Evaluation, Food and Drug Administration, Washington, DC 20204.) Journal of virological methods, **(1994 Jan)** 46 (1) 11-21. Journal code: 8005839. ISSN: 0166-0934. Pub. country: Netherlands. Language: English.
AB An improved procedure to recover and **purify** double-stranded RNA (dsRNA) from fecal specimens is described. Guanidine isothiocyanate, **hydroxyapatite**, and cetyltrimethylammonium bromide were used to extract and **purify** rotavirus dsRNA from fecal specimens. The method is very efficient and easy to perform, and precludes the use of toxic substances such as phenol, chloroform, and Freon. It yields RNA free of enzymatic inhibitors, permitting its detection by reverse transcription-polymerase chain reaction assays. In addition, it was demonstrated that during initial clarification of the fecal suspension, the pellet must be washed at least twice to avoid massive losses of **virus**, **viral** protein, or **viral** nucleic acid retained in the solid debris.

L24 ANSWER 25 OF 101 MEDLINE on STN
94002663. PubMed ID: 8399763. [New site site-specific endonuclease and methylase from Bacillus licheniformis 736]. Novye sait-spetsificheskie endonukleaza i metilaza iz Bacillus licheniformis 736. Matvienko N N; Kramarov V M; Zheleznaia L A; Matvienko N I. Biokhimiia (Moscow, Russia), **(1993 Aug)** 58 (8) 1139-53. Journal code: 0372667. ISSN: 0320-9725. Pub. country: RUSSIA: Russian Federation. Language: Russian.
AB The site-specific endonuclease R Bli736I and methylase M Bli736I have been isolated from the Bacillus licheniformis strain 736 by blue-agarose, **hydroxyapatite**-Ultragel and heparin-Sepharose chromatography. The enzymes are free from interfering impurities. R Bli736I recognizes the 5'-GGTCTCN-3' decreases and decreases 5'-NNNNNGAGACC-3' sequences on the DNA and cleaves the DNA as indicated by arrows to form single-stranded 4-nucleotide 5'-protruding termini. This enzyme is an isoschizomer of Eco3II isolated from E. coli.

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L24 ANSWER 26 OF 101 MEDLINE on STN
93354294. PubMed ID: 8350878. [Isolation and properties of BstBSI restriction endonuclease from the thermophilic soil bacteria Bacillus stearothermophilus BS]. . Vydelenie i svoistva endonukleazy restriktsii BstBSI iz termofil'noipochvennoi bakterii Bacillus stearothermophilus BS. Kovalevskaia N P; Ivanov L Iu; Zheleznaia L A; Matvienko N I. Molekuliarnaia genetika, mikrobiologiia i virusologiia, **(1993 May-Jun)** (3) 22-5. Journal code: 9315607. ISSN: 0208-0613. Pub. country: RUSSIA: Russian Federation. Language: Russian.
AB A new restriction endonuclease BstBSI was isolated and **purified** from the thermophilic soil bacterium Bacillus stearothermophilus BS by the blue sepharose and **hydroxyapatite** chromatographies. The enzyme is an isoschizomer of SnaI from Sphaerotilus natans C. It recognizes the hexanucleotide GTATAC and cleaves DNA in the center of the sequence. The maximal catalytic activity of the endonuclease is registered in 50 mM tris-HCl (pH 9.0) buffer with the high ionic strength (100 mM NaCl) in the presence of 10 mM MgCl2 at 45 degrees C. Glucosylated DNA of the phage T4 is not cleaved by the enzyme.

L24 ANSWER 27 OF 101 MEDLINE on STN
93091730. PubMed ID: 1668797. Adsorption and preparation of human **viruses** using **hydroxyapatite** column. Tsuru S; Shinomiya N; Katsura Y; Uwabe Y; Noritake M; Rokutanda M. (Department of Microbiology, National Defense Medical College, Saitama, Japan.) Bio-medical materials and engineering, **(1991)** 1 (3) 143-7. Journal code: 9104021. ISSN: 0959-2989. Pub. country: United States. Language: English.
AB The adsorption and chromatographic properties of **hydroxyapatite** sorbents for application to different **viruses** have been investigated. The strong adsorption of **viruses** was observed on macroporous **hydroxyapatite** with hydrophilic properties of the sorbent surface. The **viruses** were

purified on this sorbent without loss of biological activity. The column can be used for **virus** vaccine production.

L24 ANSWER 28 OF 101 MEDLINE on STN
93089020. PubMed ID: 1456017. Advances in osteogenin and related bone morphogenetic proteins in bone induction and repair. Luyten F P; Cunningham N S; Vukicevic S; Paralkar V; Ripamonti U; Reddi A H. (Bone cell biology section, National Institute of Dental Research, National Institutes of Health, Bethesda, MD 20892.) Acta orthopaedica Belgica, **(1992)** 58 Suppl 1 263-7. Journal code: 2985165R. ISSN: 0001-6462. Pub. country: Belgium. Language: English.
AB Bone matrix is a repository of growth and differentiation factors as demonstrated by the induction of local cartilage and bone formation in rats. The bone inductive activity, termed osteogenin, can be dissociatively extracted, and it was isolated by heparin affinity, **hydroxyapatite** and molecular sieve chromatography. Osteogenin has been **purified** to homogeneity from bovine bone matrix and the sequences of several tryptic peptides have been determined. The sequences were similar to portions of the amino acid sequence deduced from the cDNA clone of bone morphogenetic protein-3 (BMP-3). The carboxyl-terminal quarter of osteogenin has sequence identity to the corresponding regions of two related proteins BMP-2A and BMP-2B. The bone inductive proteins are members of the TGF-beta superfamily, by **virtue** of the location of the highly conserved cysteines in their carboxyl-terminal region. Osteogenin and related BMPs initiate cartilage and bone formation in vivo. The study of the mechanism of action of these proteins will add considerable new information on the molecular signals controlling endochondral bone formation. In vitro data indicate that osteogenin stimulates the expression of the osteogenic and chondrogenic phenotypes. Our results demonstrate their profound influence on proteoglycan synthesis and degradation in bovine cartilage explant cultures. High affinity specific binding sites have been identified in both MC3T3 cells and articular chondrocytes. In vivo experiments demonstrate the efficacy of primate osteogenin in restoring large calvarial defects in adult baboons, establishing a primary role for osteogenin in therapeutic initiation and promotion of osteogenesis.

L24 ANSWER 29 OF 101 MEDLINE on STN
91244982. PubMed ID: 1645368. Detection of group B and C rotaviruses by polymerase chain reaction. Gouvea V; Allen J R; Glass R I; Fang Z Y; Bremont M; Cohen J; McCrae M A; Saif L J; Sinarachatanant P; Caul E O. (Division of Viral and Rickettsial Diseases, Centers for Disease Control, Atlanta, Georgia 30333.) Journal of clinical microbiology, **(1991 Mar)** 29 (3) 519-23. Journal code: 7505564. ISSN: 0095-1137. Pub. country: United States. Language: English.
AB We adapted the polymerase chain reaction (PCR) to detect the noncultivatable group B and C rotaviruses and introduced a simple and convenient technique to **purify viral** RNA from stool specimens. Double-stranded RNA present in stool extracts was **purified** by adsorption to **hydroxyapatite** and was used as the template for reverse transcription and polymerase amplification. Primer pairs specific for group B (gene 8) and group C (gene 6) rotaviruses were selected to amplify group-characteristic sizes of cDNA copies readily identifiable in ethidium bromide-stained agarose gels. These primer pairs were used separately in individual PCR assays or were pooled with a primer pair specific for group A rotavirus (gene 9) in a combined PCR assay for the simultaneous detection of all three rotavirus groups. The method was very sensitive and was used to identify both human and porcine strains of group B and C rotaviruses in stool specimens. A second PCR amplification with internal group-specific primers served to increase further the sensitivity of the test and to confirm the diagnostic results obtained in the first amplification.

L24 ANSWER 30 OF 101 MEDLINE on STN
91160571. PubMed ID: 2001711. Immunology, biosynthesis and in vivo assembly of the branched-chain 2-oxoacid dehydrogenase complex from bovine kidney. Clarkson G H; Lindsay J G. (Department of Biochemistry, University of Glasgow, Scotland, UK.) European journal of biochemistry / FEBS, **(1991 Feb 26)** 196 (1) 95-100. Journal code: 0107600. ISSN: 0014-2956. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.
AB Specific, polyclonal antisera have been raised to the native branched-chain 2-oxoacid dehydrogenase complex (BCOADC) from bovine kidney and each of its three constituent enzymes: E1, the substrate-specific 2-oxoacid dehydrogenase; E2, the multimeric dihydrolipoamide acyltransferase 'core' enzyme and E3, dihydrolipoamide dehydrogenase. **Purified** BCOADC, isolated by selective poly(ethyleneglycol) precipitation and **hydroxyapatite** chromatography, contains only traces of endogenous E3 as detected by a requirement for this enzyme in assaying overall complex activity and by immunoblotting criteria. A weak antibody

response was elicited by the E1 beta subunit relative to the E2 and E1 alpha polypeptides employing either **purified** E1 or BCOADC as antigens. Anti-BCOADC serum showed no cross-reaction with high levels of pig heart E3 indicating the absence of antibody directed against this component. However, immunoprecipitates of mature BCOADC from detergent extracts of NBL-1 (bovine kidney) or PK-15 (porcine kidney) cell lines incubated for 3-4 h in the presence of [35S]methionine contained an additional 55,000-Mr species which was identified as E3 on the basis of immunocompetition studies. Accumulation of newly synthesised [35S]methionine-labelled precursors for E2, E1 alpha and E3 was achieved by incubation of PK-15 cells for 4 h in the presence of uncouplers of oxidative phosphorylation. Pre-E2 exhibited an apparent Mr value of 56,500, pre-E1 alpha, 49,000 and pre-E3, 57,000 compared to subunit Mr values of 50,000, 46,000 and 55,000, respectively, for the mature polypeptides. Thus, like the equivalent lipoate acyltransferases of the mammalian pyruvate dehydrogenase (PDC) and 2-oxoglutarate dehydrogenase (OGDC) complexes, pre-E2 of BCOADC characteristically contains an extended presequence. In NBL-1 cells, pre-E2 was found to be unstable since no cytoplasmic pool of this precursor could be detected; moreover, processed E1 alpha was not assembled into intact BCOADC as evidenced by the absence of E2 or E3 in immunoprecipitates with anti-(BCOADC) serum after a 45-min 'chase' period in the absence of uncoupler. Dihydrolipoamide dehydrogenase (E3), in its precursor state, was not present in immune complexes with anti-(BCOADC) serum, indicating that its co-precipitation with mature complex is by **virtue** of its high affinity for assembled complex in vivo whereas no equivalent interaction of pre-E3 with its companion precursors occurs prior to mitochondrial import.

L24 ANSWER 31 OF 101 MEDLINE on STN

91077215. PubMed ID: 1369279. A human hybrid hybridoma producing a bispecific monoclonal antibody that can target tumor cells for attack by Pseudomonas aeruginosa exotoxin A. Honda S; Ichimori Y; Iwasa S. (Research and Development Division, Takeda Chemical Industries, Ltd., Osaka, Japan.) Cytotechnology, **(1990 Jul)** 4 (1) 59-68. Journal code: 8807027. ISSN: 0920-9069. Pub. country: Netherlands. Language: English.

AB By fusing a human hybridoma producing an IgG2 kappa antibody against human A431 epidermoid carcinoma cells with an Epstein-Barr **virus**-transformed human B lymphocyte producing an IgG2 kappa antibody against Pseudomonas aeruginosa exotoxin A, we established a hybrid hybridoma producing a bispecific monoclonal antibody reacting with both A431 cells and the exotoxin. Human IgG was **purified** from the culture supernatant of the hybrid hybridoma, and the bispecific monoclonal antibody in the IgG preparation was further separated from the two parental antibodies by **hydroxyapatite** high-performance liquid chromatography. The human bispecific monoclonal antibody thus obtained efficiently targeted the antibody-reactive cells, A431, for attack by the exotoxin in vitro.

L24 ANSWER 32 OF 101 MEDLINE on STN

90307739. PubMed ID: 2195030. Expression of the alpha, beta II, and gamma protein kinase C isozymes in the baculovirus-insect cell expression system. **Purification** and characterization of the individual isoforms. Burns D J; Bloomenthal J; Lee M H; Bell R M. (Department of Biochemistry, Duke University Medical Center, Durham, North Carolina 27710.) Journal of biological chemistry, **(1990 Jul 15)** 265 (20) 12044-51. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Detailed in vitro comparisons of the biochemical characteristics of three protein kinase C isozymes were performed. As an alternative to earlier uncertain separation methods and expression schemes, highly **purified** and genetically distinct protein kinase C enzymes were produced using the baculovirus expression system. The baculovirus expression system yielded approximately 200-300 micrograms of the **purified** isozyme from 3 x 10(8) (100 ml of culture medium) baculovirus-infected insect cells. Biochemical characterization of the expressed isozymes indicated that the three isozymes had **virtually** indistinguishable Ca2+, Mg2+, and ATP dependencies. However, in certain critical functional characteristics such as phosphatidylserine dependencies, phospholipid and substrate preferences, and arachidonic acid activation, the gamma isozyme exhibited distinctive properties when compared with both the alpha and beta II subtypes. In addition, the activity of the beta II subtype was more dependent upon diacylglycerol or phorbol esters for activation than either the alpha or gamma isoforms. The alpha isozyme, unlike the beta II and gamma forms, was totally dependent on Ca2+ for activation in the presence of free arachidonic acid. These studies provide definitive characterizations of the pure isoforms; many of the findings were consistent with earlier enzymatic observations using **hydroxyapatite-purified** isoforms. Thus, the distinctive biochemical properties of the protein kinase C isozymes are consistent with the hypothesis that each isoform may have distinct roles in signal transduction processes.

L24 ANSWER 33 OF 101 MEDLINE on STN

90279505. PubMed ID: 1693746. LamB (maltoporin) of Salmonella typhimurium: isolation, **purification** and comparison of sugar binding with LamB of Escherichia coli. Schulein K; Benz R. (Lehrstuhl fur Biotechnologie, Universitat Wurzburg, FRG.) Molecular microbiology, **(1990 Apr)** 4 (4) 625-32. Journal code: 8712028. ISSN: 0950-382X. Pub. country: ENGLAND: United Kingdom. Language: English.

AB LamB (maltoporin) of Salmonella typhimurium was found to be more strongly associated with the murein than OmpF. It was **purified** in one step using a **hydroxyapatite** (HTP) column. Reconstitution of the pure protein with lipid bilayer membrane showed that LamB of S. typhimurium formed small ion-permeable channels with a single channel conductance of about 90 pS in 1 M KCl and some preference for cations over anions. The conductance concentration curve was linear, which suggested that LamB of S. typhimurium does not contain any binding site for ions. Pore conductance was completely inhibited by the addition of 20 mM maltotriose. Titration of the LamB-induced membrane conductance with different sugars, including all members of the maltooligosaccharide series up to seven glucose residues, suggested that the channel contains, like LamB (maltoporin) of Escherichia coli, a binding site for sugars. The binding constant of sugars of the maltooligosaccharide series increased with increasing number of glucose residues up to five (saturated). Small sugars had a higher stability constant for sugar binding relative to LamB of E. coli. The advantage of a binding site inside a specific porin for the permeation of solutes is discussed with respect to the properties of a general diffusion porin.

L24 ANSWER 34 OF 101 MEDLINE on STN

90203608. PubMed ID: 2108211. Neutrophil activation by inflammatory microcrystals of monosodium urate monohydrate utilizes pertussis toxin-insensitive and -sensitive pathways. Terkeltaub R A; Sklar L A; Mueller H. (VA Medical Center, Department of Medicine, San Diego, CA 92161.) Journal of immunology (Baltimore, Md. : 1950), **(1990 Apr 1)** 144 (7) 2719-24. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB The activation of leukocytes by particulates is a critical event in certain inflammatory syndromes, including acute gout associated with microcrystals of monosodium urate monohydrate. In this study we have evaluated mechanisms of human neutrophil activation by urate crystals. Both N-formyl-nor-leu-leu-phe-nor-leu-tyr-lys and uncoated urate crystals (0.5 to 5 mg/ml) but not urate crystals coated with human low density lipoprotein (which suppresses crystal-induced neutrophil responsiveness), stimulated pertussis toxin (PT)-sensitive GTPase activity in **purified** preparations of human neutrophil membranes. **Hydroxyapatite** crystals (up to 5 mg/ml) were inactive. Pretreatment of neutrophil membranes with cholera toxin also inhibited crystal-induced and formylated peptide-induced GTPase activity. Pretreatment of whole neutrophils with PT resulted in nearly complete inhibition of formylated peptide-induced cytosolic calcium mobilization, release of superoxide and release of the azurophil granule constituent alpha-mannosidase. In contrast, identical pretreatment of whole neutrophils with PT only partially suppressed urate crystal-induced alpha-mannosidase and superoxide release and failed to inhibit crystal phagocytosis and increases in cytosolic free calcium. Mechanisms of neutrophil activation by monosodium urate crystals appear to be heterogeneous in comparison with activation by formylated peptides and there is no absolute requirement for PT-sensitive membrane G proteins in neutrophil responsiveness to urate crystals.

L24 ANSWER 35 OF 101 MEDLINE on STN

89229186. PubMed ID: 2540828. Activation of rat liver cytosolic phosphatidic acid phosphatase by nucleoside diphosphates. Berglund L; Bjorkhem I; Angelin B; Einarsson K. (Department of Clinical Chemistry, Karolinska Institutet, Huddinge University Hospital, Sweden.) Biochimica et biophysica acta, **(1989 Apr 26)** 1002 (3) 382-7. Journal code: 0217513. ISSN: 0006-3002. Pub. country: Netherlands. Language: English.

AB Phosphatidic acid phosphatase (EC 3.1.3.4) was **purified** 30-fold by ammonium sulfate fractionation and **hydroxyapatite** chromatography from the soluble fraction of rat liver. ADP was found to stimulate the enzyme activity with half-maximal stimulation at 0.2 mM. Similar effects were seen when ADP was replaced by GDP or CDP. In contrast, ATP inhibited the enzyme; half-maximal inhibition observed at 0.2 mM. Again, the degree of inhibition did not differ when GTP or CTP replaced ATP. Thus, the structure of the base part of the nucleotide was not critical for mediating these effects. The positions of the phosphate groups in the nucleotide structure were however found to be of importance for the enzyme activity. Variations in the structure of the phosphate ester bound at the 5'-position had a pronounced effect on phosphatidic acid phosphatase activity. The effect of nucleotides depended on pH, and the inhibition by ATP was more pronounced at pH levels lower than 7.0, whereas the

stimulatory effect of ADP was **virtually** the same from pH 6.0 to pH 8.0. The enzyme showed substrate saturation kinetics with respect to phosphatidic acid, with an apparent Km of 0.7 mM. Km increased in the presence of ATP, whereas both apparent Vmax and Km increased in the presence of ADP, suggesting different mechanisms for the action of the two types of nucleotides. The results indicated that physiological levels of nucleotides with a diphosphate or a triphosphate ester bound at the 5'-position of the ribose moiety influenced the activity of phosphatidic acid phosphatase. The possibility is discussed that these effects might be of importance for the regulation of triacylglycerol biosynthesis.

L24 ANSWER 36 OF 101 MEDLINE on STN

89180922. PubMed ID: 2853272. Proteolysis of V antigen from Yersinia pestis. Brubaker R R; Sample A K; Yu D Z; Zahorchak R J; Hu P C; Fowler J M. (Department of Microbiology and Public Health, Michigan State University, East Lansing 48824-1101.) Microbial pathogenesis, **(1987 Jan)** 2 (1) 49-62. Journal code: 8606191. ISSN: 0882-4010. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Lcr-plasmids of yersiniae are known to mediate a unique low calcium response characterised by restriction of growth in vitro with induction of putative **virulence** factors including yersiniae outer membrane-peptides (YOPs) and V antigen (Lcr+). A medium was developed that permitted expression of high yields of V by Yersinia pestis KIM in large fermenter vessels. Immunoblots of specific precipitates prepared by prior molecular sieving showed that native unaggregated V exists as a monomeric 37,000 dalton peptide. Fractionation by precipitation with (NH4)2SO4 and chromatography on phenyl-Sepharose, DEAE cellulose, Sephacryl S200, calcium **hydroxyapatite**, and Sephadex G200 yielded highly **purified** antigen as judged by sodium dodecyl sulfate-polyacrylamide gel electrophoresis of parallel preparations from Lcr+ and Lcr- yersiniae. However, yields of V obtained by this process were unexpectedly low. As determined from immunoblots with monospecific polyclonal and monoclonal anti-V, this loss of activity occurred as a function of evident degradation at every step of **purification** yielding antigenic fragments of about 36,000, 34,000, 31,000, 30,000, and 28,000 daltons. Neutral or acidic pH favored hydrolysis; insignificant cleavage occurred in viable Lcr+ cells of Y. pestis or in culture supernatant fluids. V in neutral cytoplasm from Yersinia pseudotuberculosis or Yersinia enterocolitica did not undergo comparable degradation.

L24 ANSWER 37 OF 101 MEDLINE on STN

89078612. PubMed ID: 2462512. Pyruvate kinase type M2 is phosphorylated at tyrosine residues in cells transformed by Rous sarcoma **virus**. Presek P; Reinacher M; Eigenbrodt E. (Rudolf Buchheim-Institut fur Pharmakologie, Justus Liebig-Universitat Giessen, FRG.) FEBS letters, **(1988 Dec 19)** 242 (1) 194-8. Journal code: 0155157. ISSN: 0014-5793. Pub. country: Netherlands. Language: English.

AB Chicken embryo cells (CECs) contain pyruvate kinase (PK) type M2 (M2-PK). Transformation of CECs by Rous sarcoma **virus** (RSV) leads to a reduction in the affinity of PK for the substrate phosphoenolpyruvate. In vitro, M2-PK can be phosphorylated at tyrosine residues by pp60v-src, the transforming protein of RSV. To study tyrosine phosphorylation of M2-PK in intact RSV-transformed cells, the protein was immunoprecipitated from 32P-labeled normal and RSV-SR-A-transformed CECs. Phosphoamino acid analysis of immunoprecipitated M2-PK revealed that M2-PK of both normal and transformed CECs contained phosphoserine and small amounts of phosphothreonine. Only M2-PK of transformed CECs contained phosphotyrosine in addition. For enzyme kinetic studies M2-PK was partially **purified** by chromatography upon DEAE-Sephacel and **hydroxyapatite**. A decreased affinity for phosphoenolpyruvate was observed 3 h after the onset of transformation using the temperature-sensitive mutant of RSV, ts-NY 68. The kinetic changes were correlated with tyrosine phosphorylation of M2-PK, but there is no direct evidence that they are caused by post-translational modification of the enzyme.

L24 ANSWER 38 OF 101 MEDLINE on STN

89073761. PubMed ID: 3201752. **Purification** and characterization of vaccinia **virus** structural protein VP8. Yang W P; Bauer W R. (Department of Biochemistry, School of Medicine, State University of New York, Stony Brook 11794.) Virology, **(1988 Dec)** 167 (2) 578-84. Journal code: 0110674. ISSN: 0042-6822. Pub. country: United States. Language: English.

AB A major vaccinia **virus** core protein, designated VP8, has been **purified** from **virions** to homogeneity through DEAE-cellulose, CM-cellulose, and **hydroxyapatite** chromatography. VP8 migrates as a 25-kDa band in SDS-polyacrylamide gel electrophoresis and sediments as a monomeric species in neutral sucrose gradient centrifugation. This protein is a significant constitutent of the **virion**, comprising about 6.5% of the total **viral** polypeptides by mass. Analysis by filter binding and by

sucrose gradient centrifugation shows that VP8 binds to double-stranded as well as to single-stranded DNA at low salt concentrations (25 mM NaCl). At higher salt concentrations (100 mM NaCl), the protein binds with a relatively greater affinity to single-stranded DNA. The results from sucrose gradient centrifugation indicate that VP8 probably binds noncooperatively to all structural forms of DNA. The protein is likely to be a component of the **viral** nucleoprotein complex.

L24 ANSWER 39 OF 101 MEDLINE on STN

88292944. PubMed ID: 2840854. The isolation and **purification** of pre-S2 containing hepatitis B **virus** surface antigen by chemical affinity chromatography. Lin J Y; Hsieh Y S; Chu S C. (Institute of Biochemistry, College of Medicine, National Taiwan University, Taipei, R.O.C.) Applied biochemistry and biotechnology, **(1987 Oct)** 15 (3) 255-63. Journal code: 8208561. ISSN: 0273-2289. Pub. country: United States. Language: English.

AB A simple, rapid, and efficient method was developed to isolate and **purify** pre-S2 containing HBsAgs from the plasma of a single chronic carrier of HBsAg (adw) by ammonium sulfate fractionation, **hydroxyapatite** column chromatography, and polymerized human serum albumin-affinity column chromatography. About 500 micrograms of pre-S2 containing HBsAg was obtained from 140 mL of plasma containing 4,200 micrograms of HBsAg. Two **purified** pre-S2 containing HBsAgs were analyzed by SDS-polyacrylamide gel electrophoresis and their molecular weights were determined to be 31,000 and 68,000 respectively. No significant amount of HBsAg or its derivative was detected in the final product.

L24 ANSWER 40 OF 101 MEDLINE on STN

88044486. PubMed ID: 3314115. Crystallization, enzymatic cleavage, and the polarity of the **adenovirus** type 2 fiber. Devaux C; Caillet-Boudin M L; Jacrot B; Boulanger P. (European Molecular Biology Laboratory, Grenoble Outstation, France.) Virology, **(1987 Nov)** 161 (1) 121-8. Journal code: 0110674. ISSN: 0042-6822. Pub. country: United States. Language: English.

AB Crystals of the fiber protein of **adenovirus** type 2 have been grown. Analysis of these crystals (type I crystals) showed that they were composed of fiber polypeptide with a lower apparent molecular weight (60 kDa) than that of the soluble or **virion**-incorporated fiber (62 kDa). N-terminal sequencing revealed that the fiber polypeptide chain of 60 kDa was cleaved at tyrosine17 from the N-end. The C-terminus remained intact. Assays with protease inhibitors suggested that the spontaneous cleavage of the fiber occurring upon its crystallization was due to a cellular, calcium-dependent, chymotrypsin-like protease co-**purifying** with the fiber and activated during **hydroxyapatite** chromatography. Crystallization of fiber **purified** in the presence of chymostatin provided crystals of a different structure under the electron microscope (crystals of type II), composed of 62-kDa fiber polypeptide units. The 62-kDa fiber from the type II crystals, as well as the 62-kDa fiber isolated from infected cell extracts, were able to associate with the penton base in vitro to form a penton capsomer. The 60-kDa fiber has lost this capacity. The accessibility of the N- and C-termini of the fiber inside the penton structure was probed by anti-peptide sera after limited proteolysis. The results are consistent with a polarity of the fiber in which its N-terminus is oriented toward the penton base, the C-terminal domain corresponding to the distal knob.

L24 ANSWER 41 OF 101 MEDLINE on STN

87322574. PubMed ID: 2442971. Isolation and characterization of mutants of Streptococcus mutans using selective removal of wild-type cells by agglutination with an agglutinin from Persea americana. Curtiss R 3rd; Pearce C; Pollack J; Murchison H M. Acta microbiologica Polonica, **(1987)** 36 (1-2) 3-15. Journal code: 7610362. ISSN: 0137-1320. Pub. country: Poland. Language: English.

AB Persea americana agglutinin (PAA), a substance known to bind basic proteins and inhibit the sucrose-independent adherence of Streptococcus mutants to saliva = coated **hydroxyapatite** (Staat et al., 1980) was used to selectively enrich for mutants defective in a variety of cell surface associated **virulence** characteristics from cultures UAB62 (PS14 Riff, serotype c), UAB66 (6715 Strr Spcr, serotype g) and UAB77 (GS5, serotype c). Following mutagenesis and growth for segregation and phenotypic expression, washed cells of each strain were exposed to PAA overnight at 37 degrees C. Aggregated cells were removed by low-speed centrifugation and cells remaining in the supernatant fluids were concentrated, grown to stationary phase and the enrichment with PAA repeated. Mutants isolated following enrichment were phenotypically diverse and included strains defective in one or more of the following characteristics: adherence to glass in a sucrose-containing medium, aggregation with sucrose, dextran or PAA. dextranase production, colony morphology, cell or chain morphology, fermentation of sorbitol, lactose, galactose, raffinose, melibiose, or fructose, and production of surface protein antigen A (SpaA). The diversity of mutant phenotypes identified along with the observation that

PAA could still cause aggregation (with a lower efficiency) of all mutants leads us to infer that the interaction of this agglutinin with proteins on the S. mutans cell surface is relatively nonspecific and that the observed inhibition of S. mutants attachment to saliva-coated **hydroxyapatite** caused by PAA is not due to a highly specific unique interaction of PAA with the protein(s) responsible for sucrose-independent adherence.

L24 ANSWER 42 OF 101 MEDLINE on STN

87273542. PubMed ID: 3111722. Identification of a major 50-kDa molecular weight human B-cell growth factor with Tac antigen-inducing activity on B cells. Kawano M; Matsushima K; Oppenheim J J. Cellular immunology, **(1987 Aug)** 108 (1) 132-49. Journal code: 1246405. ISSN: 0008-8749. Pub. country: United States. Language: English.

AB A bioassay was developed using human small B cells adherent to anti-human IgM (anti-mu)-coated wells. These B cells were stimulated to proliferate by culture supernatants of concanavalin A (Con A)-activated human peripheral blood lymphocytes (Con A Sup) even in the presence of high concentrations of anti-mu coated on assay wells. Human B-cell growth factor (BCGF) activities were partially **purified** from Con A Sup. Preparative chromatography (Sephacryl S-200 and isoelectrofocusing) yielded a major peak of BCGF activity for B cells adherent to anti-mu-coated wells with a molecular weight of 50,000 (50 kDa) and a pI 7.6. The 50-kDa BCGF was further **purified** by sequential chromatography using DEAE-Sephacel, CM-Sepharose, Sephacryl S-200, CM-high performance liquid chromatography (HPLC), and **hydroxyapatite** (HA)-HPLC. The HA-HPLC-**purified** 50-kDa BCGF was free of interleukin-1 (IL-1), interleukin-2 (IL-2), and interferon activities, but could support growth of BCL1 cells, similar to BCGF-II. Neither IL-1 nor interferon-gamma had any growth-stimulating effect in our B-cell proliferation assay with or without BCGF in Iscove's synthetic assay medium. BCGF-induced proliferation of B cells adherent to anti-mu-coated wells could be markedly augmented by the simultaneous or sequential addition of recombinant human IL-2 (rIL-2). When cultured for 3 days with 50-kDa BCGF, about 40% of B cells adherent to anti-mu-coated wells expressed Tac antigen, and monoclonal anti-Tac antibody inhibited rIL-2 enhancement of proliferation of 50-kDa BCGF-preactivated B cells. In addition, 50-kDa BCGF could induce Tac antigen on an Epstein-Barr **virus**-transformed B-cell line (ORSON) in the presence of a suboptimal dose of phorbol myristate acetate (PMA) and also on a natural killer-like cell line (YT cells). We have therefore identified a major 50-kDa BCGF activity with Tac antigen-inducing activity that also has a synergistic effect with IL-2 on normal B-cell proliferation.

L24 ANSWER 43 OF 101 MEDLINE on STN

87154340. PubMed ID: 3826625. Rapid **purification** of Bordetella pertussis toxin by alternating affinity and hydrophobic chromatography. Svoboda M; Hannecart-Pokorni E; Borremans M; Christophe J. Analytical biochemistry, **(1986 Dec)** 159 (2) 402-11. Journal code: 0370535. ISSN: 0003-2697. Pub. country: United States. Language: English.

AB Pertussis toxin was **purified** to homogeneity from a 2-day culture supernatant of Bordetella pertussis by stepwise elution from three columns of, consecutively, Blue Sepharose, phenyl Sepharose, and **hydroxyapatite**. The toxin was eluted from Blue Sepharose and **hydroxyapatite** by high ionic strength and from phenyl Sepharose with low ionic strength and with 17% glycerol. Toxin fractions from one chromatographic column were immediately charged on the next column, saving laborious and time-consuming concentration or dialysis steps. Based on peptide composition (after sodium dodecyl sulfate-polyacrylamide gel electrophoresis) and on HPLC profile (under nondenaturing conditions), the toxin was already practically pure after two steps, the third **hydroxyapatite** column serving only to separate the whole native toxin from any free S1 subunit. Recovery was estimated from the capacity of the preactivated toxin (and any preexisting free S1 subunit) to catalyze the ADP-ribosylation of the guanine nucleotide binding protein Ni in rat pancreatic plasma membranes: of the total capacity initially present in the culture medium, 23% could be recovered as pure native toxin with the present procedure. Besides, the nondenaturing HPLC method used to check the purity of the native toxin appeared to be superior to classical acidic polyacrylamide gel electrophoresis.

L24 ANSWER 44 OF 101 MEDLINE on STN

87085493. PubMed ID: 2432165. Functional **purification** and enzymic characterization of the RNA-dependent DNA polymerase of human immunodeficiency **virus**. Wondrak E M; Lower J; Kurth R. Journal of general virology, **(1986 Dec)** 67 (Pt 12) 2791-7. Journal code: 0077340. ISSN: 0022-1317. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The RNA-dependent DNA polymerase (RDDP) of human immunodeficiency **virus** (HIV) was **purified** from sucrose density gradient-banded **virus** by four successive procedures: anion exchange chromatography, cation exchange

chromatography, affinity chromatography on oligo(dT)-cellulose and adsorption chromatography on **hydroxyapatite**. The enzyme preparation was free of cellular DNA-dependent DNA polymerase activity. The properties of HIV RDDP were determined with a variety of template-primers. Generally, the enzyme used Mg2+ for optimal activity except with (Cm)n X (dG)12-18 as template-primer. Kinetic data (Michaelis constant, Hill coefficient) were calculated for several substrates.

L24 ANSWER 45 OF 101 MEDLINE on STN
86304229. PubMed ID: 2427504. Isolation and characterization of alpha-macroglobulin from guinea pig plasma. Suzuki Y; Sinohara H. Journal of biochemistry, **(1986 Jun)** 99 (6) 1655-65. Journal code: 0376600. ISSN: 0021-924X. Pub. country: Japan. Language: English.
AB Guinea pig alpha-macroglobulin was **purified** to apparent homogeneity by sequential chromatography on Sephacryl S-300, DEAE-cellulose, and **hydroxyapatite**. A molecular weight of 780,000 was obtained by equilibrium sedimentation. The preparation migrated as a single band of Mr = 180,000 in sodium dodecyl sulfate-polyacrylamide gel electrophoresis under reducing conditions. Rabbit antiserum raised against the final preparation partially cross-reacted with human and rat alpha-2-macroglobulins but not with rat alpha-1-macroglobulin. Guinea pig alpha-macroglobulin stimulated the amidolytic activity of trypsin towards a small substrate, but inhibited the proteolytic activity of trypsin towards remazol brilliant blue hide powder. When treated with trypsin or methylamine, four thiol groups per molecule were newly generated. The reaction with trypsin proceeded with at least at two different rates: half of the thiol groups were generated in a fast reaction and the remaining half in a slower reaction. On the other hand, such a two-step reaction was not detected in the reaction with methylamine. The methylamine-treated alpha-macroglobulin retained half the capacity to bind trypsin and its mobility in polyacrylamide gel under nondenaturing conditions remained **virtually** unchanged. These properties are in marked contrast to those reported for human alpha-2-macroglobulin, but resemble those of rat alpha-2- and mouse alpha-macroglobulins. The amidase activity of trypsin bound to guinea pig alpha-macroglobulin was impaired by soybean trypsin inhibitor to a much greater degree than that of trypsin bound to human or rat alpha-2-macroglobulin.

L24 ANSWER 46 OF 101 MEDLINE on STN
86108335. PubMed ID: 3080314. Isolation and properties of porcine lecithin:cholesterol acyltransferase. Knipping G. European journal of biochemistry / FEBS, **(1986 Jan 15)** 154 (2) 289-94. Journal code: 0107600. ISSN: 0014-2956. Pub. country: GERMANY, WEST: Germany, Federal Republic of. Language: English.
AB Lecithin: cholesterol acyltransferase (LCAT, phosphatidylcholine: sterol O-acyltransferase, EC 2.3.1.43) was **purified** approximately 20 000-fold from pig plasma by ultracentrifugation, phenyl-Sepharose and **hydroxyapatite** chromatography. **Purified** LCAT had an apparent relative molecular mass of 69 000 +/- 2000. By isoelectrofocusing it separated into five or six bands with pI values ranging from pH 4.9 to 5.2. The amino acid composition was similar to that of the human enzyme. An antibody against pig LCAT was prepared in goat. The antibody reacted against pig LCAT and gave a reaction of partial identity with human LCAT. Incubation of pig plasma or **purified** enzyme with the antibody **virtually** inhibited LCAT activity. The same amount of antibody inactivated only 62% of the LCAT activity in human serum. Pig and human LCAT were activated to the same extent by either human or pig apolipoprotein A-I (apo-A-I) using small liposomes as substrate. Human apoA-I, however, caused a higher esterification rate for both enzymes. Using apoA-I and small liposomes as a substrate, the addition of apoC-II up to 4 micrograms/ml had no effect on the LCAT reaction, but above this concentration LCAT was inhibited. Small liposomes with phosphatidylcholine/cholesterol molar ratios of 3:1 up to 8.4:1 did not show any significant differences in the LCAT reaction, when used as substrates in the presence of various amounts of apoA-I and albumin. In contrast, the LCAT activity was significantly reduced by liposomes with phosphatidylcholine/cholesterol molar ratios below 3:1.

L24 ANSWER 47 OF 101 MEDLINE on STN
86062922. PubMed ID: 2999443. Proteolytic cleavage of the E2 glycoprotein of murine coronavirus: activation of cell-fusing activity of **virions** by trypsin and separation of two different 90K cleavage fragments. Sturman L S; Ricard C S; Holmes K V. Journal of virology, **(1985 Dec)** 56 (3) 904-11. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.
AB In the murine coronavirus mouse hepatitis **virus**, a single glycoprotein, E2, is required both for attachment to cells and for cell fusion. Cell fusion induced by infection with mouse hepatitis **virus** strain A59 was inhibited by the addition of monospecific anti-E2 antibody after **virus**

adsorption and penetration. Adsorption of concentrated coronavirions to uninfected cells did not cause cell fusion in the presence of cycloheximide. Thus, cell fusion was induced by E2 on the plasma membrane of infected 17 Cl 1 cells but not by E2 on **virions** grown in these cells. Trypsin treatment of **virions purified** from 17 Cl 1 cells quantitatively cleaved 180K E2 to 90K E2 and activated cell-fusing activity of the **virions**. This proteolytic cleavage yielded two different 90K species which were separable by sodium dodecyl sulfate-**hydroxyapatite** chromatography. One of the trypsin cleavage products, 90A, was acylated and may be associated with the lipid bilayer. The other, 90B, was not acylated and yielded different peptides than did 90A upon limited digestion with thermolysin or staphylococcal V8 protease. Thus, the cell-fusing activity of a coronavirus required proteolytic cleavage of the E2 glycoprotein, either by the addition of a protease to **virions** or by cellular proteases acting on E2, which was transported to the plasma membrane during **virus** maturation. There is a striking functional similarity between the E2 glycoprotein of coronavirus, which is a positive-strand RNA **virus**, and the hemagglutinin glycoprotein of negative-strand orthomyxoviruses, in that a single glycoprotein has both attachment and protease-activated cell-fusing activities.

L24 ANSWER 48 OF 101 MEDLINE on STN

86004718. PubMed ID: 4043087. Flavobacterium heparinum 6-O-sulphatase for N-substituted glucosamine 6-O-sulphate. Bruce J S; McLean M W; Williamson F B; Long W F. European journal of biochemistry / FEBS, **(1985 Oct 1)** 152 (1) 75-82. Journal code: 0107600. ISSN: 0014-2956. Pub. country: GERMANY, WEST: Germany, Federal Republic of. Language: English.

AB A specific glyco-6-O-sulphatase has been **purified** to homogeneity from Flavobacterium heparinum. The enzyme hydrolyses the 6-O-sulphates of 2-deoxy-2-sulphamido-6-O-sulpho-D-glucose (GlcNS-6S), 2-acetamido-2-deoxy-6-O-sulpho-D-glucose (GlcNAc-6S) and 2-amino-2-deoxy-6-O-sulphato-D-glucose (GlcN-6S). The activity was **purified** 2100-fold by successive chromatography on CM-Sepharose CL-6B, Sepharose CL-4B, **hydroxyapatite** and blue-Sepharose CL-6B. Sodium dodecyl sulphate/polyacrylamide gel electrophoresis showed a protein of relative molecular mass 64000. Four novel assays were developed using 35S-labelled and 14C-labelled monosaccharides. The **purified** enzyme was free of all other known heparin-degrading enzymes. In particular this was the first resolution of the 6-O-sulphatase from the sulphamidase. Optimal activity was at pH 7.5. Enzyme activity was **virtually** unaffected by Na+ and K+ ions. Enhancements of activity of 12% and 30% were effected by Mg2+ and Ca2+ ions respectively. Inorganic phosphate and sulphate (both 0.005 mol dm-3) inhibited activity by 48% and 50% respectively. The Km value for the free amino substrate GlcN-6S was 1.35 mmol dm-3. In contrast the Km values for the GlcNAc-6S and GlcNS-6S were 54 mumol dm-3 and 16 mumol dm-3 respectively.

L24 ANSWER 49 OF 101 MEDLINE on STN

85289634. PubMed ID: 2993328. Isolation of the subunits of the coronavirus envelope glycoprotein E2 by **hydroxyapatite** high-performance liquid chromatography. Ricard C S; Sturman L S. Journal of chromatography, **(1985 Jun 19)** 326 191-7. Journal code: 0427043. ISSN: 0021-9673. Pub. country: Netherlands. Language: English.

AB The coronavirus glycoprotein E2, which is responsible for **virus** attachment to cell receptors and **virus**-induced cell fusion, was **purified** by solubilization of **virions** with Triton X-114 and phase fractionation. Native E2 and tryptic subunits of the glycoprotein were separated by size-exclusion high-performance liquid chromatography (HPLC). Two distinct 90 kD E2 subunits, which had identical electrophoretic mobilities when analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, were separated by **hydroxyapatite** HPLC in the presence of sodium dodecyl sulfate.

L24 ANSWER 50 OF 101 MEDLINE on STN

85179492. PubMed ID: 3987694. Flavobacterium heparinum 3-O-sulphatase for N-substituted glucosamine 3-O-sulphate. Bruce J S; McLean M W; Long W F; Williamson F B. European journal of biochemistry / FEBS, **(1985 Apr 15)** 148 (2) 359-65. Journal code: 0107600. ISSN: 0014-2956. Pub. country: GERMANY, WEST: Germany, Federal Republic of. Language: English.

AB A novel bacterial sulphatase has been discovered in an extract of Flavobacterium heparinum. The enzyme hydrolyses the 3-O-sulphate from 2-deoxy-2-sulphamido-3-O-sulpho-D-glucose and 2-acetamido-2-deoxy-3-O-sulpho-D-glucose. The activity was **purified** 10 800-fold by chromatography successively on CM-Sepharose CL-6B, **hydroxyapatite**, taurine-Sepharose CL-4B and CM-Sepharose CL-6B. Sodium dodecylsulphate/polyacrylamide gel electrophoresis showed the enzyme to be homogeneous and of relative molecular mass 56 000. Two novel assays were developed using 2-[14C]acetamido-2-deoxy-3-O-sulpho-D-glucose and 2-deoxy-2-sulphamido-3-O-sulpho-D-glucose as respective substrates. The

purified 3-O-sulphatase was shown to be free of all other known heparin-degrading enzymes. Optimal activity was at pH 7.5 for the disulphated substrate and pH 8.0 for the N-acetylated substrate. Enzyme activity was **virtually** unaffected by Na+, K+ or Mg2+ ions. A 1.2-fold enhancement of activity was effected by 0.002 mol dm-3 Ca2+. Inorganic phosphate and sulphate inhibited 3-O-sulphatase activity. The Km value of the N-acetylated substrate was determined to be 42 mumol dm-3. No activity was detected with 2-amino-2-deoxy-3-O-sulpho-D-glucose.

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L24 ANSWER 51 OF 101 MEDLINE on STN
85158060. PubMed ID: 6099371. **Purification** of the structural and nonstructural proteins of St. Louis encephalitis **virus**. Vorndam A V; Trent D W. Journal of virological methods, **(1984 Dec)** 9 (4) 283-91. Journal code: 8005839. ISSN: 0166-0934. Pub. country: Netherlands. Language: English.
AB We have developed a procedure for **purifying** both the structural and nonstructural proteins of flaviviruses from lysates of infected cell cultures. The procedure involves: immunoprecipitation to concentrate **viral** proteins and eliminate most of the cellular proteins, preparative polyacrylamide gel electrophoresis to separate the **viral** proteins, and **hydroxyapatite** chromatography, which eliminates most of the unlabeled cellular protein. This procedure offers an improvement over previous **purification** schemes in that there is no loss of **viral** proteins after the immunoprecipitation step, any combination of labeling isotopes may be used, and it is not necessary to soak proteins out of gel slices.

L24 ANSWER 52 OF 101 MEDLINE on STN
85154571. PubMed ID: 2984046. 'Red pigment' from ADE-2 mutants of S. cerevisiae prevents DNA cleavage by restriction endonucleases. Meskauskas A; Ksenzenko V; Shlyapnikov M; Kryukov V; Citavicius D. FEBS letters, **(1985 Mar 25)** 182 (2) 413-4. Journal code: 0155157. ISSN: 0014-5793. Pub. country: Netherlands. Language: English.
AB Protection of DNA from cleavage by restriction endonucleases EcoRI, HindIII, BamHI, and Bg/II with red pigment, produced by ADE-2 mutants of Saccharomyces cerevisiae is demonstrated. **Purification** of yeast DNA from pigment can be achieved by chromatography on **hydroxyapatite** columns.

L24 ANSWER 53 OF 101 MEDLINE on STN
85058193. PubMed ID: 6094828. Mammalian DNA enriched for replication origins is enriched for snap-back sequences. Zannis-Hadjopoulos M; Kaufmann G; Martin R G. Journal of molecular biology, **(1984 Nov 15)** 179 (4) 577-86. Journal code: 2985088R. ISSN: 0022-2836. Pub. country: ENGLAND: United Kingdom. Language: English.
AB Using the instability of replication loops as a method for the isolation of double-stranded nascent DNA, extruded DNA enriched for replication origins was obtained and denatured. Snap-back DNA, single-stranded DNA with inverted repeats (palindromic sequences), reassociates rapidly into stem-loop structures with zero-order kinetics when conditions are changed from denaturing to renaturing, and can be assayed by chromatography on **hydroxyapatite**. Origin-enriched nascent DNA strands from mouse, rat and monkey cells growing either synchronously or asynchronously were **purified** and assayed for the presence of snap-back sequences. The results show that origin-enriched DNA is also enriched for snap-back sequences, implying that some origins for mammalian DNA replication contain or lie near palindromic sequences.

L24 ANSWER 54 OF 101 MEDLINE on STN
84160757. PubMed ID: 6200446. **Purification** of a specific inhibitor of reverse transcriptase from human placenta. Leong J C; Wood S O; Lyford A O; Levy J A. International journal of cancer. Journal international du cancer, **(1984 Apr 15)** 33 (4) 435-9. Journal code: 0042124. ISSN: 0020-7136. Pub. country: Denmark. Language: English.
AB Human placental extracts contain a factor which specifically and reversibly inhibits the reverse transcriptase of mammalian **retroviruses**. This placental inhibitor has been partially **purified** and characterized. It elutes at 0.1-0.2 M phosphate on **hydroxyapatite** chromatography and can be further **purified** by phosphocellulose chromatography where it elutes at 0.4 M KCl. By these **purification** procedures, specific activities of 40-70,000 units of inhibitor per mg of protein were obtained. The size of the inhibitor is about 60-65,000 daltons as estimated by velocity sedimentation. The inhibitor **purified** by these techniques selectively inhibits the activity of **purified** reverse transcriptase from Rauscher murine leukemia **virus** and baboon endogenous **virus**. It is substantially less active against the reverse transcriptase of avian myeloblastosis **virus**. The specificity of this inhibitor for mammalian enzymes and particularly for the human placental

reverse transcriptase suggests that it plays a role in the regulation of DNA synthesis in human placental development.

L24 ANSWER 55 OF 101 MEDLINE on STN
84154707. PubMed ID: 6704398. ATP(GTP)-dependent conversion of MVM parvovirus single-stranded DNA to its replicative form by a **purified** 10 S species of mouse DNA polymerase alpha. Faust E A; Gloor G; MacIntyre M F; Nagy R. Biochimica et biophysica acta, **(1984 Apr 5)** 781 (3) 216-24. Journal code: 0217513. ISSN: 0006-3002. Pub. country: Netherlands. Language: English.
AB A species of DNA polymerase alpha that is active in the ATP(GTP)-dependent conversion of MVM parvovirus single-stranded linear DNA to the duplex replicative form has been **purified** 4300-fold from Ehrlich ascites mouse tumour cells. The single-stranded----replicative form activity is maintained throughout ammonium sulfate precipitation, DEAE-cellulose, phosphocellulose and **hydroxyapatite** column chromatography and glycerol gradient sedimentation. Polypeptides with Mr = 230 000, 220 000, 183 000, 157 000, 125 000, 70 000, 65 000, 62 000, 57 000, 53 000 and 48 000 copurify with the single-stranded----replicative form activity, which sediments at approx. 10 S. The Mr = 183 000, 157 000 and 125 000 polypeptides exhibit catalytic activity when assayed in situ following SDS-polyacrylamide gel electrophoresis. The 10 S form of DNA polymerase alpha is functionally distinguishable from an 8.4 S form of the enzyme obtained from the same cells on the basis of single-stranded---- replicative form activity. The single-stranded----replicative form activity of the 10 S enzyme is stable at 22 degrees C for up to 3 h, but exhibits a half life of only 5 min at 45 degrees C.

L24 ANSWER 56 OF 101 MEDLINE on STN
84032875. PubMed ID: 6313727. Electron microscopic studies of transcriptional complexes released from vaccinia cores during RNA-synthesis in vitro: methods for fractionation of transcriptional complexes. Esteban M; Cabrera C V; Holowczak J A. Journal of virological methods, **(1983 Aug)** 7 (2) 73-92. Journal code: 8005839. ISSN: 0166-0934. Pub. country: Netherlands. Language: English.
AB Electron microscopic (EM) and biochemical methods were employed to study the transcriptional complexes present in detergent lysates of vaccinia **virus** cores actively synthesizing RNA in vitro. When processed and examined in the EM, 14 'transcriptional sites' could be observed on full-length DNA templates. Fractionation of lysates by equilibrium density centrifugation in CsSO4, chromatography on **hydroxyapatite** columns or by sedimentation in sucrose gradients, allowed isolation of DNA templates associated with transcripts but these manipulations often resulted in fragmentation of the DNA template or promoted the release of transcripts from the template. It is suggested that RNA transcripts remain associated with the template in regions of supercoiling. These regions, in turn, may be maintained by DNA-protein interactions which are compromised as the transcriptional complexes are fractionated and **purified**.

L24 ANSWER 57 OF 101 MEDLINE on STN
83138809. PubMed ID: 6761443. Evolution of single-copy DNA and the ADH gene in seven drosophilids. Zwiebel L J; Cohn V H; Wright D R; Moore G P. Journal of molecular evolution, **(1982)** 19 (1) 62-71. Journal code: 0360051. ISSN: 0022-2844. Pub. country: GERMANY, WEST: Germany, Federal Republic of. Language: English.
AB Single-copy DNA was isolated from Drosophila melanogaster and hybridized with total genomic DNA of D. melanogaster, D. mauritiana, D. simulans, D. pseudoobscura, D. willistoni, D. hydei and D. **virilis**. The duplexes were thermally eluted from **hydroxyapatite** and the data used to assess the relatedness of each species to D. melanogaster. The general pattern of relatedness was similar to that predicted by morphological methods but with some notable exceptions. The rate of nucleotide substitution was estimated to be greater than 0.66% of bases per million years. An unexpected, rapidly evolving component of D. melanogaster single-copy DNA was identified. The relatedness of these species was also studied with respect to the gene coding for alcohol dehydrogenase (ADH). The ADH gene, previously cloned from D. melanogaster (Goldberg 1980), was hybridized with Southern blots of genomic digests of the seven species. The intensity and position of the hybridizing bands suggest the amount of divergence of the gene. Divergence was quantitated by reassociation of a fragment of the cloned ADH gene with total DNA of the seven drosophilids and thermal elution of the resultant duplexes from **hydroxyapatite**. The ADH gene was isolated from genomic clone libraries of D. melanogaster, D. simulans and D. mauritiana and further studied by comparison of position of restriction sites. Species relationships deduced from comparison of total single-copy DNA and the ADH gene were consistent, demonstrating that a single gene can reflect divergence of the entire genome.

L24 ANSWER 58 OF 101 MEDLINE on STN
83023215. PubMed ID: 7126596. Circular dichroism and biochemical properties of the hepatitis B **virus** core antigen. Yamaki M; Ohori H; Onodera S; Ishida N; Maeda H. Biochimica et biophysica acta, **(1982 Sep 7)** 706 (2) 165-73. Journal code: 0217513. ISSN: 0006-3002. Pub. country: Netherlands. Language: English.

AB The hepatitis B **virus** (HBV) core antigen was **purified** by mild procedures, including **hydroxyapatite** column chromatography, with care taken to avoid the degradation of the particles. Circular dichroism (CD) of the HBV core particles in saline showed low intensities of negative ellipticities in the region dominated by amide bond absorption. Acid treatment of the particles induced a remarkable change in the CD spectrum, with the appearance of a positive extremum at about 208 nm. The amino acid composition and the COOH-terminal residue of the isolated core polypeptide (Mr 21,000-21,500) were shown to be essentially the same as those of the polypeptide deduced from the nucleotide sequences which had been proposed for the HBV core antigen by other laboratories. We failed to detect any NH2-terminal dansyl-derivatives from the core polypeptide by the dansyl-Edman method. We also showed by the method of fluorescein polarization that the core polypeptide conjugated with fluorescein isothiocyanate has an affinity for serum albumin. This may indicate a state of disassembled or non-assembled core polypeptide in sera.

L24 ANSWER 59 OF 101 MEDLINE on STN
82242344. PubMed ID: 7097863. **Purification** of a native membrane-associated **adenovirus** tumor antigen. Persson H; Katze M G; Philipson L. Journal of virology, **(1982 Jun)** 42 (3) 905-17. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB A 15,000-dalton protein was **purified** from HeLa cells infected with **adenovirus** type 2. Proteins solubilized from a membrane fraction of lytically infected cells was used as the starting material for **purification**. Subsequent **purification** steps involved lentil-lectin, phosphocellulose, **hydroxyapatite**, DEAE-cellulose, and aminohexyl-Sepharose chromatographies. A monospecific antiserum, raised against the **purified** protein, immunoprecipitated a 15,000-dalton protein encoded in early-region E1B (E1B/15K protein) of the **adenovirus** type 2 DNA. Tryptic finger print analysis revealed that the **purified** protein was identical to the E1B/15K protein encoded in the transforming part of the **viral** genome. The antiserum immunoprecipitated the E1B/15K protein from a variety of **viral** transformed cell lines isolated from humans, rats, or hamsters. The E1B/15K protein was associated with the membrane fraction of both lytically and **virus**-transformed cell lines and could only be released by detergent treatment. Furthermore, a 11,000- to 12,000-dalton protein that could be precipitated with the anti-E1B/15K serum was recovered from membranes treated with trypsin or proteinase K, suggesting that a major part of the E1B/15K protein is protected in membrane vesicles. Translation of early **viral** mRNA in a cell-free system, supplemented with rough microsomes, showed that this protein was associated with the membrane fraction also in vitro.

L24 ANSWER 60 OF 101 MEDLINE on STN
82192587. PubMed ID: 6281469. Organization of type C **viral** DNA sequences endogenous to baboons: analysis with cloned **viral** DNA. Battula N; Hager G L; Todaro G J. Journal of virology, **(1982 Feb)** 41 (2) 583-92. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Unintegrated linear and circular forms of baboon endogenous type C **virus** M7 DNA were prepared from M7-infected cells by chromatography on **hydroxyapatite** columns, and the circular DNAs were **purified** in cesium chloride-ethidium bromide equilibrium density gradients. The circular DNAs were linearized by digestion with EcoRI, which had a unique site on the **viral** DNA. The linearized DNA was then inserted into lambda gtWES. lambda B at the EcoRI site and cloned in an approved EK2 host. Molecularly cloned full-length M7 DNA was restricted with BamHI, and the resulting five subgenomic fragments were then subcloned individually in plasmid pBR322. The organization and sites of integration of the approximately 100 copies of M7 DNA sequences endogenous to baboons were investigated by digesting the DNA with restriction enzymes and identifying the **virus**-specific fragments by hybridization to labeled probes made by using the molecularly cloned full-length and subgenomic fragments of the **viral** DNA. We found that most of the endogenous sequences had sizes and organizations similar to those of the unintegrated **viral** DNA and therefore approximately similar to the RNA of the infectious **virus**. A few of the multiple sequences had deletions in the 3' end (envelope region), and some of the sequences either lacked or contained modified BamHI restriction sites on the 5' end of the **viral** DNA. The endogenous **viral** DNA sequences were nontandem, uninterrupted, and colinear with the DNA of the infectious **virus**, and they were integrated at different sites

in the baboon DNA, like the M7 proviral DNA sequences acquired upon infection.

L24 ANSWER 61 OF 101 MEDLINE on STN
82072740. PubMed ID: 7030741. Pyruvate dehydrogenase complex from baker's yeast. 2. Molecular structure, dissociation, and implications for the origin of mitochondria. Kresze G B; Ronft H. European journal of biochemistry / FEBS, **(1981 Oct)** 119 (3) 581-7. Journal code: 0107600. ISSN: 0014-2956. Pub. country: GERMANY, WEST: Germany, Federal Republic of. Language: English.
AB 1. Pyruvate dehydrogenase complex from Saccharomyces cerevisiae is similar in size (s20,w 77 S) and flavin content (1.3--1.4 nmol/mg) to the complexes from mammalian mitochondria. 2. The relative molecular masses of the constituent polypeptide chains, as determined by dodecylsulfate gel electrophoresis at different gel concentrations, were: lipoate acetyltransferase (E2), 58 000; lipoamide dehydrogenase (E3), 56 000; pyruvate dehydrogenase (E1), alpha-subunit, 45 000, and beta-subunit, 35 000. Gel chromatography in the presence of 6 M guanidine . HCl gave a value of 52 000 for E2 indicating anomalous electrophoretic migration as described for the E2 components of other pyruvate dehydrogenase complexes. Thus, the organization and subunit Mr values are similar with the mammalian complexes and **virtually** identical with the complexes of gram-positive bacteria but differ greatly from the pyruvate dehydrogenase complexes of gram-negative bacteria. 3. The complex was resolved into its component enzymes by the following methods. E1 was obtained by treatment of the complex with elastase followed by gel chromatography on Sepharose CL-2B using a reverse ammonium sulfate gradient for elution. E2 was isolated by gel filtration of the complex in the presence of 2 M KBr, and E3 was obtained by **hydroxyapatite** chromatography in 8 M urea. The isolated enzymes reassociated spontaneously to give pyruvate dehydrogenase overall activity.

L24 ANSWER 62 OF 101 MEDLINE on STN
81247236. PubMed ID: 7255356. Improved rapid methodology for the isolation of nucleic acids from agarose gels. Tracy S. Preparative biochemistry, **(1981)** 11 (3) 251-68. Journal code: 1276634. ISSN: 0032-7484. Pub. country: United States. Language: English.
AB Improved methodology is presented with which DNA may be rapidly isolated from agarose gels. **Hydroxyapatite** is used to bind the nucleic acid after agarose solubilization and a sodium citrate buffer is used to elute the nucleic acid free of agarose. Rapid concentration of the sample may then be effected by ethanol precipitation. **Purified** oyster glycogen may be used as carrier in this regard and does not inhibit restriction endonucleases nor T4 DNA ligase in the concentrations used. This methodology is useful for the isolation of single-and double-stranded DNA, supercoil plasmid DNA, and mRNA.

L24 ANSWER 63 OF 101 MEDLINE on STN
81168066. PubMed ID: 6783635. Isolation and characterization of sex-steroid-binding protein from rat and rabbit plasma. Suzuki Y; Ito M; Sinohara H. Journal of biochemistry, **(1981 Jan)** 89 (1) 231-6. Journal code: 0376600. ISSN: 0021-924X. Pub. country: Japan. Language: English.
AB The sex-steroid-binding plasma protein (SBP), which had been reported to be absent in the rat by several workers, was detected in 6-week-old male rats, but not in female rats aged 5-43 days. The rat SBP was **purified** to apparent homogeneity by affinity chromatography on testosterone-17-alpha-ethynylcarboxyaminoethyl Sepharose 4B followed by **hydroxyapatite** column chromatography, and its properties were compared with those of rabbit SBP prepared in exactly the same fashion. The concentration of SBP in adult male rabbit was 25-fold greater than that in 6-week-old male rat, but the binding characteristics of both SBPs were very similar. They specifically bind testosterone, 5 alpha-dihydrotestosterone, and 5 alpha-androstane-3 alpha, 17 beta-diol, but have **virtually** no affinity for estradiol, progesterone, or cortisol. Equilibrium dissociation constants for dihydrotestosterone were estimated to be 11.7 nM for rat SBP and 14.9 nM for rabbit SBP.

L24 ANSWER 64 OF 101 MEDLINE on STN
81076570. PubMed ID: 6255415. The extraction by micrococcal nuclease of glucocorticoid receptors and mouse mammary tumor **virus** DNA sequences is dissociated. Andre J; Raynaud A; Rochefort H. Nucleic acids research, **(1980 Aug 11)** 8 (15) 3393-411. Journal code: 0411011. ISSN: 0305-1048. Pub. country: ENGLAND: United Kingdom. Language: English.
AB Glucocorticoid receptors (RG) and mammary tumor **virus** (MM-TV) DNA sequences were extracted by micrococcal nuclease digestion from the nuclei of C3H mouse mammary tumor cells in order to specify their relative distribution in chromatin. RG was labelled and translocated into the nuclei by incubating cells with 3H Dexamethasone (3H Dex). The **purified** nuclei were then treated at 2 degrees C with micrococcal nuclease. Three

chromatin fractions were successively obtained: an isotonic extract (ne3H1), ahypotonic extract (ne2) and the residual pellet (P). The Dex-RG complexes were measured by the **hydroxyapatite** technique. The MMTV DNA sequences were titrated by molecular hybridization with an excess of MMTV radioactive cDNA probe. Up to 75% of the nuclear 3H Dex and the MMTV radioactive cDNA probe. Up to 75% of the nuclear 3H Dex and MMTV DNA sequences were extracted in a concentration dependent manner while only 10-15% of nucleic acids became soluble in 10% perchloric acid. The extracted 3H Dex-RG complex was found to be partly bound to soluble chromatin and partly free. The free complex displayed similar sedimentation constants (4S, 7S) and DNA binding ability to the cytosol receptor. The 3H Dex-RG complexes were 2 to 8 fold more concentrated in ne1, which is known to be enriched in active chromatin, than in ne2. Conversely, the concentration of MMTV DNA sequences per microgram DNA was the same in the three nuclear fractions. These results suggest that the Dex-RG complexes are concentrated in an active fraction of chromatin. We propose that, among the 20-30 copies of MMTV genes per haploid genome, only a small proportion are transcribed or regulated.

L24 ANSWER 65 OF 101 MEDLINE on STN

81071202. PubMed ID: 7441207. Spectrophotometric characteristics of cholera phage phi2 DNA. Chaudhuri K; Maiti M. Journal of general virology, **(1980 Aug)** 49 (2) 433-6. Journal code: 0077340. ISSN: 0022-1317. Pub. country: ENGLAND: United Kingdom. Language: English.

AB **Purified** preparations of cholera bacteriophage phi2 were treated with cold phenol and the nucleic acid examined for its **hydroxyapatite** chromatographic pattern, thermal denaturation profile, base composition and mol. wt.

L24 ANSWER 66 OF 101 MEDLINE on STN

81028277. PubMed ID: 6158579. **Purification** and biochemical characterization of the Epstein-Barr **virus**-determined nuclear antigen and an associated protein with a 53,000-dalton subunit. Luka J; Jornvall H; Klein G. Journal of virology, **(1980 Sep)** 35 (3) 592-602. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB The Epstein-Barr **virus**-determined nuclear antigen (EBNA) was **purified** 700-fold to apparent homogeneity from Raji and Namalwa cell extracts by a three-step procedure involving heat treatment, DNA-cellulose chromatography, and **hydroxyapatite** chromatography. Acid-fixed nuclear binding and complement fixation were used to monitor antigenic specificity. **Purified** EBNA was also capable of specifically inhibiting the regular anticomplement immunofluorescence reaction for EBNA against Raji target cells. The **purified** antigen had a molecular weight of 170,000 to 200,000. By sodium dodecyl sulfate-polyacrylamide gel electrophoresis, it yielded a single 48,000-dalton (48K) monomer. An EBNA-associated protein was also **purified** from the same cell extract. It had a molecular weight of about 200,000 and yielded a single 53K protein band by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The same protein was also found in Epstein-Barr **virus** negative B-cell lymphoma lines. The two types of protein were characterized by amino acid composition and peptide mapping. The results showed that the 53K and 48K protein components have no long regions in common; this excludes that the smaller product arises by breakdown of the larger product. Residue distributions were different, but an excess of hydrophilic residues was found in both proteins, suggesting a certain overall similarity in properties. 53K components from different cell lines appeared to differ somewhat. Epstein-Barr **virus**-positive lines carry two 53K components, one of which may be a slightly modified 53K product. Immunocomplexing assay showed that the 48K, but not the 53K, protein carries EBNA specificity. In mixtures, the 53K protein is co-precipitated with the 48K protein. The data suggest that EBNA may form a complex with the 53K proten within the cell.

L24 ANSWER 67 OF 101 MEDLINE on STN

80248411. PubMed ID: 7399676. Fibril-mediated adherence of Actinomyces viscosus to saliva-treated **hydroxyapatite**. Wheeler T T; Clark W B. Infection and immunity, **(1980 May)** 28 (2) 577-84. Journal code: 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.

AB Fibril-mediated adherence of Actinomyces viscosus strain T14V cells to saliva-treated **hydroxyapatite** was studied. Fibrils were **purified** by ammonium sulfate precipitation and differential centrifugation from the crude supernatant of whole cells that were sheared by one passage through a French pressure cell. **Purified** fibrils and crude supernatant inhibited strain T14V adherence to saliva-treated **hydroxyapatite** to similar extents. However, anti-strain T14V serum and antifibril specific antibody completely abolished strain T14V adherence. The blocking immunoglobulin could be adsorbed from anti-T14V serum by strain T14V whole cells, by **purified** fibrils, and, to a lesser extent, by cell walls. It

was concluded that fibrils mediate adherence of strain T14V cells to saliva-treated **hydroxyapatite**. In addition, fibril preparations were shown to contain more than 95% protein and to be antigenically homogeneous by immunodiffusion and Laurell rocket immunoelectrophoresis. **Purified** fibril preparations showed serological identity with the **virulence**-associated 1 antigen of Lancefield-extracted T14V cells, whereas crude supernatants contained both **virulence**-associated 1 and **virulence**-associated 2 antigens, as shown by rocket immunoelectrophoresis.

L24 ANSWER 68 OF 101 MEDLINE on STN
80221173. PubMed ID: 6248116. Physical properties of the complementary T4 RNA. Helland D E. Biochimica et biophysica acta, **(1980 Jun 27)** 608 (1) 127-37. Journal code: 0217513. ISSN: 0006-3002. Pub. country: Netherlands. Language: English.
AB The complementary transcribed T4 RNA after self-annealing and RNAase treatment was isolated by gel chromatography and then used for further studies. From salt-dependent RNAase resistance and melting studies it is evident that this RNA represents a genuine double-stranded structure. The base content of the isolated double-stranded RNA was found to be the same as total T4 mRNA. Sucrose gradient analysis and **hydroxyapatite** chromatography of T4 RNA, annealed early and late RNA, and of the isolated double-stranded RNA, gave results indicating that the complementary RNA is part of a RNA molecule and further that the size of the complementary regions are independent of the RNA molecules. Partial digestion of pulse-labelled late RNA with phosphodiesterase I prior to annealing with unlabelled early RNA, showed that the complementary regions on the mRNA are not located to the 5'- or 3'-end but randomly distributed along the T4 RNA molecules.

L24 ANSWER 69 OF 101 MEDLINE on STN
80164759. PubMed ID: 232213. The isolation of extrachromosomal DNA by **hydroxyapatite** chromatography. Shoyab M; Sen A. Methods in enzymology, **(1979)** 68 199-206. Journal code: 0212271. ISSN: 0076-6879. Pub. country: United States. Language: English.

L24 ANSWER 70 OF 101 MEDLINE on STN
80065640. PubMed ID: 508758. **Hydroxyapatite** chromatography of short single-stranded DNA. Wittelsberger S C; Hansen J N. Biochimica et biophysica acta, **(1979 Nov 22)** 565 (1) 125-30. Journal code: 0217513. ISSN: 0006-3002. Pub. country: Netherlands. Language: English.
AB Short single-stranded segments of calf thymus DNA were obtained by random cleavage with DNAase I. After treatment with various concentrations of DNAase I, fragment sizes were estimated using the ratio of total to terminal phosphorus. DNA populations ranging from 4--180 bases were obtained. Fragments with lengths up to 1140 were generated by shearing in a **Virtis** homogenizer. The **hydroxyapatite** elution profiles of sized populations were determined by elution with phosphate gradients. A curve relating elution molarities to single-strand chain length was 'biphasic', with the elution molarity being extremely sensitive to chain lengths below 50 nucleotides but much less sensitive to chain lengths above 100 nucleotides. These results show that single-stranded fragments below 50 nucleotides elute from **hydroxyapatite** appreciably before high molecular-weight denatured DNA using phosphate gradients. This is an important consideration when using **hydroxyapatite** to fractionate DNA populations which contain short single strands.

L24 ANSWER 71 OF 101 MEDLINE on STN
80034994. PubMed ID: 291053. Isolation and characterization of germ line DNA from mouse sperm. Shiurba R; Nandi S. Proceedings of the National Academy of Sciences of the United States of America, **(1979 Aug)** 76 (8) 3947-51. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.
AB Mouse germ line DNA was isolated from sperm by a physicochemical procedure that preferentially destroys contaminating somatic cell DNA. The use of reducing conditions and chelating agents in combination with phenol permitted extraction of molecular weight DNA from mature sperm nuclei with approximately 80% efficiency. Less than 0.1% somatic cell DNA contamination remained in sperm DNA prepared by this method. Germ line DNA was characterized by determination of its ultraviolet absorbance spectrum, buoyant density in cesium chloride, and melting profile on a **hydroxyapatite** column. Contamination by mitochondrial DNA was assessed by cesium chloride/ethidium bromide gradient centrifugation. The significance of the mouse germ line DNA isolation procedure is discussed with respect to the possible genetic transmission of mammary tumor **virus** and leukemia **virus**, the origin of antibody diversity, and the origin of testicular teratomas.

L24 ANSWER 72 OF 101 MEDLINE on STN
80021071. PubMed ID: 90522. Characterization of an RNA-directed

DNA-polymerase from a cell line derived from a radiation-induced lymphoma in mice. Sarin P S; Donlon J; Friedman B; Gallo R C. Biochimica et biophysica acta, **(1979 Sep 27)** 564 (2) 235-45. Journal code: 0217513. ISSN: 0006-3002. Pub. country: Netherlands. Language: English.

AB An RNA-directed DNA polymerase was **purified** from a cell line derived from a radiation-induced lymphoma in NIH Swiss mice which produced non-infectious type C **virus** particles. The enzyme was isolated from a high speed particulate fraction which bands at a density of 1.16--1.19 g/ml in a sucrose gradient, and **purified** by successive chromatography on DEAE-cellulose, phosphocellulose and **hydroxyapatite**. The **purified** DNA polymerase has a molecular weight of 68 000, a pH optimum of 7.5, a KCl optimum of 50 mM, and a Mn2+ optimum of 0.25 mM. It prefers (dT)15 . (A)n to (dT)15 . (dA)n as the primer template and transcribes the poly(C) strand of (dG)15 .(C)n and (dG)15 . (OMeC)n. It transcribes heteropolymeric regions of avian myeloblastosis **virus** 70 S RNA, and is inhibited by antiserum to Rauscher murine leukemia **virus** DNA polymerase. Comparison of the properties of DNA polymerase **purified** from radiation-induced lymphoma cells with the DNA polymerase **purified** from non-defective murine type C RNA tumor **viruses** shows that the mouse lymphoma enzyme is both biochemically and immunologically related to murine leukemia **virus** DNA polymerases.

L24 ANSWER 73 OF 101 MEDLINE on STN

79193978. PubMed ID: 109427. **Purification** and characterization of testosterone-binding globulin of canine serum. Suzuki Y; Okumura Y; Sinohara H. Journal of biochemistry, **(1979 May)** 85 (5) 1195-203. Journal code: 0376600. ISSN: 0021-924X. Pub. country: Japan. Language: English.

AB Testosterone-binding globulin (TeBG) of canine serum was **purified** to apparent homogeneity by affinity chromatography on testosterone-17 alpha-ethynylcarboxyaminoethyl-Sepharose 4B followed by **hydroxyapatite** column chromatography. Canine TeBG was a glycoprotein containing 5.5% carbohydrates. Equilibrium sedimentation analysis in the presence and absence of 6 M guanidine hydrochloride gave molecular weights of 40,000 and 76,000, respectively, suggesting that native TeBG consists of two subunits. Equilibrium dissociation constants at 0 degrees C for testosterone and dihydrotestosterone were estimated to be 5.58 x 10(-8) M and 1.43 x 10(-8) M, respectively, and the number of binding site per native molecule was approximately unity for both androgens. Canine TeBG had **virtually** no affinity for estradiol, progesterone, or cortisol. Canine TeBG did not cross-react with a rabbit antiserum raised against bovine TeBG.

L24 ANSWER 74 OF 101 MEDLINE on STN

79187996. PubMed ID: 444534. **Purification** and properties of a nuclear DNA endonuclease from HeLa S3 cells. Fischman G J; Lambert M W; Studzinski G P. Biochimica et biophysica acta, **(1979 Apr 12)** 567 (2) 464-71. Journal code: 0217513. ISSN: 0006-3002. Pub. country: Netherlands. Language: English.

AB An endonuclease that can act on calf thymus DNA and circular doublestranded phage PM2 DNA has been isolated from HeLa S3 cell chromatin. Approximately 200-fold **purification** was achieved by a sequence of subcellular fractionation, differential NaCl solubility and chromatography on CM-Sephadex, DEAE-cellulose and **hydroxyapatite**, and isoelectric point is pH 5.1 +/- 0.2. Divalent cations are necessary for its activity and the enzyme is heat inactivated at 60 degrees C. The enzyme activity is sensitive to caffeine and sulfhydryl reacting compounds. The molecular weight, determined by gel filtration and SDS gel electrophoresis, is approx. 22 000.

L24 ANSWER 75 OF 101 MEDLINE on STN

79103926. PubMed ID: 216411. Properties and **purification** of a glucose-regulated protein from chick embryo fibroblasts. Shiu R P; Pastan I H. Biochimica et biophysica acta, **(1979 Jan 25)** 576 (1) 141-50. Journal code: 0217513. ISSN: 0006-3002. Pub. country: Netherlands. Language: English.

AB A glucose-regulated protein of molecular weight 78,000 (GRP-78) had been **purified** from a membrane fraction isolated from **viral** transformed chick embryo fibroblasts. **Purification** was achieved by extraction of the membrane fraction with Triton X-100, and chromatography on diethylaminoethyl-cellulose and **hydroxyapatite**. The **purified** protein exhibited one single spot on two-dimensional polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate and has a pI of about 5.3. A monospecific antiserum to GRP-78 was generated in a goat. Immunofluorescence studies using affinity **purified** antibodies to GRP-78 revealed that this protein was not exposed on the cell surface but was localized in a granular vesicular network inside the cell that resembles the distribution of endoplasmic reticulum. The availability of **purified** GRP-78 and a specific antiserum to it should prove useful in elucidating

the role of this protein in glucose metabolism and its relationship to malignant transformation.

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L24 ANSWER 76 OF 101 MEDLINE on STN
79029802. PubMed ID: 212583. **Purification** of the Epstein-Barr **virus**-determined nuclear antigen from Epstein-Barr **virus**-transformed human lymphoid cell lines. Luka J; Lindahl T; Klein G. Journal of virology, **(1978 Sep)** 27 (3) 604-11. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.
AB The Epstein-Barr **virus**-determined nuclear antigen (EBNA) was **purified** from extracts of the human lymphoid cell lines Raji, Namalwa, and B95-8/MLD by two different methods. In the first approach, the apparently native antigen was **purified** 1,200-fold by a four-step procedure involving DNA-cellulose chromatography, blue dexptran-agarose chromatography, **hydroxyapatite** chromatography, and gel filtration, employing complement fixation as the assay procedure. Such EBNA preparations specifically inhibited the anticomplement immunofluorescence test for EBNA and bound to methanol/acetic acid-fixed metaphase chromosomes. The **purified** antigen, which has a molecular weight of 170,000 to 200,000, yielded a single protein band of molecular weight about 48,000 by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis. These data indicate that native EBNA has a tetrameric structure. In the second **purification** method, EBNA-containing cell extracts containing radioactively labeled proteins were incubated with anti-EBNA-positive sera, and antigen-antibody complexes were adsorbed to matrix-bound staphylococcal protein A. The bound proteins were then released with an SDS-containing buffer, and denatured EBNA was separated from antibody chains by SDS-polyacrylamide gel electrophoresis and visualized by fluorography. The denatured EBNA obtained in radiochemically pure form by this procedure has a molecular weight of about 48,000, so both methods yield an EBNA monomer of the same size.

L24 ANSWER 77 OF 101 MEDLINE on STN
79005639. PubMed ID: 211127. A rapid method for the **purification** of extrachromosomal DNA from eukaryotic cells. Shoyab M; Sen A. Journal of biological chemistry, **(1978 Oct 10)** 253 (19) 6654-6. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.
AB A simple and efficient procedure to **purify** the low molecular weight extrachromosomal DNA from eukaryotic cells is described. Gentle lysis of cells with urea and sodium dodecyl sulfate in 0.24 M phosphate buffer (pH 6.8) is followed by the removal of high molecular weight bulk DNA by centrifugation. Protein and RNA are removed from the supernatant by **hydroxyapatite** chromatography in urea/phosphate buffer. Urea is then removed with 0.15 M phosphate buffer and the extrachromosomal DNA, **virtually** free from protein and RNA, is finally eluted in 0.5 M phosphate buffer. The procedure allows the recovery of about 99% simian **virus** 40 (SV40) DNA from infected monkey kidney cells in the extrachromosomal fraction. In normal mouse, monkey, andhuman cells, approximately 1% of total cell DNA appears to be extrachromosomal.

L24 ANSWER 78 OF 101 MEDLINE on STN
79000383. PubMed ID: 687604. Isolation of the ADP, ATP carrier as the carboxyatractylate . protein complex from mitochondria. Klingenberg M; Riccio P; Aquila H. Biochimica et biophysica acta, **(1978 Aug 8)** 503 (2) 193-210. Journal code: 0217513. ISSN: 0006-3002. Pub. country: Netherlands. Language: English.
AB The procedure for the isolation from mitochondria of the undenatured ADP, ATP carrier is described. The condition of retaining the nativity are elaborated. 1. As indicator for the ADP, ATP carrier (35S)- or (3H) carboxyatractylate were used. By preloading the mitochondria with carboxyatractylate, a stable carboxyatractylate . protein complex could be retained after solubilization with Triton X-100. Among the polyoxyethylene detergents emulphogen is also solubilizing, whereas Brij and Lubrol fail to solubilize. 2. When unloaded mitochondria are solubilized the capacity for binding carboxyatractylate disappears rapidly, particularly at 20 degrees C. 3. When mitochondria are preloaded with atractylate, the binding after solubilization with Triton X-100 is considerably lower than with carboxyatractylate, indicating that the high affinity of carboxyatractylate is required for effectively protecting the protein. 4. For **purification hydroxyapatite** is most effective. The carboxyatractylate-protein complex appears in the pass-through whereas the bulk of other mitochondrial proteins are retained such that a 7-fold **purification** is obtained. The nonadsorptivity to **hydroxyapatite** is dependent on the undenatured state maintained in the carboxyatractylate . protein complex. 5. Subsequent gel filtration on Sepharose results in a 1.5-fold further enrichment of specific carboxyatractylate binding up to

17 mumol/g protein, corresponding to a 10-fold **purification** from mitochondria. This value cannot be increased with further measures. 6. At the last **purification** step, in sodium dodecyl sulfate polyacrylamide gel electrophoresis **virtually** a single band of 30 000 molecular weight is found, confirming the purity at this stage. A molecular weight of 60 000 is calculated from the carboxyatractylate binding, indicating that the carboxyatractylate protein complex consists of two 30 000 subunits. From this the protein share of the ADP, ATP carrier in beef heart mitochondria can be calculated to amount to 9.5%9 7. The intact carboxyatractylate . protein complex is protected against proteolytic degradation. The release of carboxyatractylate ensues a conformational change of protein as assayed by conformation specific antibodies, concomitant with unmasking of proteolytic site as assayed by tryptic digestion. 8. The amino acid composition indicates hydrophobicity (39% polarity) and a high content of basic amino acid such as lysine and arginine. There is 1.5 mol percent cysteine and a blocked N-terminal. 9. From the solubilized complex (35S) carboxyatractylate can be removed by carboxyatractylate, ADP and ATP but not by ITP, etc., indicating the presence of recognizing sites specific fof ADP, ATP and therefore, identity with the ADP, ATP carrier. 10. Other reported procedures for isolating the ADP, ATP carrier are shown to either fail or have lower yield than the present, original procedure.

L24 ANSWER 79 OF 101 MEDLINE on STN
78225521. PubMed ID: 673856. A method for the recovery of DNA from agarose gels. Tabak H F; Flavell R A. Nucleic acids research, **(1978 Jul)** 5 (7) 2321-32. Journal code: 0411011. ISSN: 0305-1048. Pub. country: ENGLAND: United Kingdom. Language: English.
AB We describe a quick and versatile method for the isolation of DNA from agarose gels. The DNA is electrophoresed into a trough containing **hydroxyapatite**, where it is bound. The **hydroxyapatite** is taken out and the DNA eluted with phosphate buffer. By putting the **hydroxyapatite** on a small column of Sephadex G50, elution and subsequent removal of phosphate can be performed in one step. The DNA recovered can be used equally well in enzymatic incubations as DNA not **purified** through agarose gel electrophoresis. Several applications of this technique are described.

L24 ANSWER 80 OF 101 MEDLINE on STN
78185001. PubMed ID: 207199. Large-scale isolation and partial **purification** of type C RNA **viruses** on **hydroxyapatite**. 1. Biochemical characterization. Smith R G; Lee S A. Analytical biochemistry, **(1978 May)** 86 (1) 252-63. Journal code: 0370535. ISSN: 0003-2697. Pub. country: United States. Language: English.

L24 ANSWER 81 OF 101 MEDLINE on STN
78167214. PubMed ID: 206358. Radioimmunoassay for protein p28 of murine mammary tumor **virus** in organs and serum of mice and search for related antigens in human sera and breast cancer extracts. Hendrick J C; Francois C; Calberg-Bacq C M; Colin C; Franchimont P; Gosselin L; Kozma S; Osterrieth P M. Cancer research, **(1978 Jun)** 38 (6) 1826-31. Journal code: 2984705R. ISSN: 0008-5472. Pub. country: United States. Language: English.
AB The main protein of the core of murine mammary tumor **virus**, with a molecular weight of 28,000 (p28), was solubilized by deoxycholate treatment of the **virus** and **purified** by Ultrogel ACA-54 filtration and **hydroxyapatite** chromatography. This protein was used as labeled antigen in a highly specific and reproducible radioimmunoassay. Organ extracts of uninfected C57BL mice did not contain p28, but organ extracts of infected RIII mice did contain the antigen. Despite the high content in the mammary gland, the level of p28 in the other organs was identical in male and female mice. Sera of uninfected mice and the majority of the sera of infected mice did not contain the antigen. The investigation included 338 human sera (50, normal; 157, breast cancer; 77, polycystic disease; 32, benign mastopathy; 12, fibroadenoma; 10, at risk of developing breast cancer). None contained an antigen related to p28. Eight of 24 extracts of human breast cancer gave results that appeared weakly positive, possibly as a result of proteolysis. Extracts of healthy breast tissue and the serum from the breast arterial and venous blood of corresponding patients were negative.

L24 ANSWER 82 OF 101 MEDLINE on STN
78089719. PubMed ID: 621785. Procedure for **purification** of intact DNA from vaccinia **virus**. Cabrera C V; Esteban M. Journal of virology, **(1978 Jan)** 25 (1) 442-5. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.
AB A procedure for the isolation of intact vaccinia DNA molecules by chromatography on **hydroxyapatite** in the presence of 6 M urea is described. When lysates of **virions** containing 0.5 to 10 microgram of DNA were employed, over 95% of the **viral** DNA could be recovered free of

poteins. Vaccinia DNA molecules isolated in this manner sedimented at 68S in neutral sucrose gradients and had an average contour length of 62.3 micrometer when examined in an electron microscope, and the DNA could be cleaved with the restriction endonuclease EcoRI and BamHI. The results of these analyses showed that intact vaccinia DNA molecules of 120 X 10(6) to 130 X 10(6) molecular weight could be obtained by the procedures described.

L24 ANSWER 83 OF 101 MEDLINE on STN
78084346. PubMed ID: 563788. Chick-embryo DNA polymerase gamma. Identity of gamma-polymerases **purified** from nuclei and mitochondria. Bertazzoni U; Scovassi A I; Brun G M. European journal of biochemistry / FEBS, **(1977 Dec 1)** 81 (2) 237-48. Journal code: 0107600. ISSN: 0014-2956. Pub. country: GERMANY, WEST: Germany, Federal Republic of. Language: English.
AB The level of DNA polymerase gamma as compared to DNA polymerases alpha and beta has been determined in chick embryo by means of specific tests: the amount of gamma-polymerase in the 12-day-old chick embryo reaches about 15% of the total polymerase activity. This enzyme is mainly localized in nuclei and mitochondria, where it represents the prevailing if not the unique DNA polymerase activity. The mitochondrial DNA polymerase gamma is likely to be associated with the internal membrane or the matrix of this organelle since it is not removed by digitonin treatment. The gamma-polymerases have been **purified** from chick embryo nuclei and mitochondria 500-700 times by means of DEAE-cellulose, phosphocellulose and **hydroxyapatite** chromatographies. The **purified** mitochondrial DNA polymerase gamma is closely related to the homologous enzyme **purified** from the nuclei of the same cells. So far, they cannot be distinguished on the basis of their sedimentation, catalytical properties and response to inhibitors or denaturating agents. The **purified** gamma enzymes are distinct from the chick embryo DNA polymerases alpha and beta and are not inhibited by antibodies prepared against the latter enzymes. The nuclear and mitochondrial gamma-polymerases do not respond to the oncogenic RNA **virus** DNA polymerase assay with natural mRNAs.

L24 ANSWER 84 OF 101 MEDLINE on STN
78044971. PubMed ID: 924490. Simple method of **purification** of bacteriophage lambda by **hydroxyapatite** column chromatography. Das S; Ghosh S. Indian journal of biochemistry & biophysics, **(1977 Mar)** 14 (1) 65-7. Journal code: 0310774. ISSN: 0301-1208. Pub. country: India. Language: English.

L24 ANSWER 85 OF 101 MEDLINE on STN
78040500. PubMed ID: 200268. **Purification** and characterization of baboon endogenous **virus** DNA polymerase. Sarin P S; Friedman B; Gallo R C. Biochimica et biophysica acta, **(1977 Nov 16)** 479 (2) 198-206. Journal code: 0217513. ISSN: 0006-3002. Pub. country: Netherlands. Language: English.
AB An RNA-directed DNA polymerase was **purified** from baboon endogenous type-C **virus** by successive column chromatography on DEAE cellulose, phosphocellulose and **hydroxyapatite**. The **purified** DNA polymerase has a molecular weight of 68 000, a pH optimum of 8.0, a Mn2+ optimum of 1 mM, and a KCl optimum of 40 mM. The **purified** enzyme transcribes heteropolymeric regions of **viral** 60--70 S RNA isolated from different type-C **viruses**. The **purified** enzyme is immunologically related to a similarly **purified** polymerase from the cat endogenous type-C **virus** RD114.

L24 ANSWER 86 OF 101 MEDLINE on STN
77065694. PubMed ID: 63292. **Purification** and characterization of gibbon ape leukemia **virus** DNA polymerase. Sarin P S; Gallo R C. Biochimica et biophysica acta, **(1976 Dec 1)** 454 (2) 212-21. Journal code: 0217513. ISSN: 0006-3002. Pub. country: Netherlands. Language: English.
AB An RNA directed DNA polymerase was **purified** over 2500 fold from gibbon ape leukemia **virus** by successive column chromatography on Sephadex G100, DEAE cellulose, phosphocellulose and **hydroxyapatite**. The **purified** DNA polymerase has a molecular weight of 68 000, a pH optimum of 7.5, a Mn2+ optimum of 0.8 mM, and KCl optimum of 80 mM. The **purified** enzyme transcribes heteropolymeric regions of **viral** 60-70 S RNA isolated from avian myeloblastosis **virus**, Rauscher murine leukemia **virus** and simian sarcoma **virus** and it is inhibited by antiserum prepared against either gibbon ape leukemia **virus** or simian sarcoma **virus** DNA polymerases.

L24 ANSWER 87 OF 101 MEDLINE on STN
77031841. PubMed ID: 185420. Isolation and structural characterization of monomeric and dimeric forms of replicative intermediates of Kilham rat **virus** DNA. Gunther M; May P. Journal of virology, **(1976 Oct)** 20 (1) 86-95. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.
AB Two **virus**-specific species of newly synthesized DNA were isolated from rat fibroblast cell cultures infected with the Kilham rat **virus** (RV).

These two DNA species were **purified**; their behavior on **hydroxyapatite** chromatography and their sedimentation coefficients in sucrose gradients were determined. One of the two species corresponds to the linear double-stranded form of the RV DNA, and the other corresponds to the dimeric duplex form. After denaturation, a fraction of both species showed an intramolecular renaturation; these molecules are composed of **viral** strand covalently linked to complementary strand. Models for the structure of both species are posposed. Both species may be considered as double-strand replicative intermediates of the single-stranded RV DNA.

L24 ANSWER 88 OF 101 MEDLINE on STN
76024145. PubMed ID: 170217. Appearance of C-type **virus**-like particles after co-cultivation of a human tumor-cell line with rat (XC) cells. Gabelman N; Waxman S; Smith W; Douglas S D. International journal of cancer. Journal international du cancer, **(1975 Sep 15)** 16 (3) 355-69. Journal code: 0042124. ISSN: 0020-7136. Pub. country: Denmark. Language: English.

AB A serially progagated cell line (L104) was established by co-cultivation of alung adenocarcinoma (L-1) from a patient with concurrent chronic lymphocytic leukemia and XC, a non-producer rat line, known to carry the Rous sarcoma **virus** (RSV) genome. Karyotype of the L104 cultures revealed predominantly rat-like patterns; however, about 5% of the cells reacted with HLA antibodies and demonstrated human isozyme patterns. Electron microscopy of L104 cells revealed the presence of C-type particles budding from the cell membranes and in cytoplasmic vacuoles. **Virus** was not detected in any of the other normal lung, lung tumor or XC cells examined after co-cultivation with XC cells. The particles isolated from tissue culture fluids had the biochemical and biophysical characteristics common to other known mammalian C-type particles and were serologically related to the woolly monkey **virus** (WMV)/gibbon ape leukemia **virus** (GaLV) complex. Cross-hybridization between **viral** 3H-DNA transcripts and cellular RNAs from **virus**-infected cells clearly show the presence of sequences in the L104 cellular RNA related to both the GaLV/WMV group of **viruses** and rat **viruses**. **Hydroxyapatite** chromatography reveals however that the primate-related sequences in the **viral** RNA are indistinguishable from WMV in thermal elution profile. The host range of L104 **virus** appears to vary greatly from WMV in being xenotropic and, in the cell lines thus far tested appears, to infect only rat cells. The **virus** gave positive KC but negative XC assays. Inoculation of whole cells or cell-free supernatants into weaning hamster did not result in either solid tumors or leukemia. Co-cultivation of appropriate cell lines may represent an approach to the detection of latent **viruses** in human neoplasia.

L24 ANSWER 89 OF 101 MEDLINE on STN
75208988. PubMed ID: 1149742. Improved techniques for the fractionation of non-histone proteins of chromatin on **hydroxyapatite**. Rickwood D; MacGillivray A J. European journal of biochemistry / FEBS, **(1975 Feb 21)** 51 (2) 593-601. Journal code: 0107600. ISSN: 0014-2956. Pub. country: GERMANY, WEST: Germany, Federal Republic of. Language: English.

AB The effect of the dissociation medium on the fractionation of chromatin on **hydroxyapatite** has been studied. Optimal separations of the histones and non-histone protein are only achieved when columns are run in buffers containing high concentrations of sodium ions. We have modified our previously published method such that the chromosomal proteins can be recovered in **virtually** quantitative yields. Each of the **hydroxyapatite** fractions has been analysed with respect to nucleic acid content and the proteins have been analysed by two-dimensional gel electrophoresis.

L24 ANSWER 90 OF 101 MEDLINE on STN
75184070. PubMed ID: 166680. A rapid **purification** method of restriction endonucleases from Haemophilus strains. Kopecka H. Biochimica et biophysica acta, **(1975 May 23)** 391 (1) 109-20. Journal code: 0217513. ISSN: 0006-3002. Pub. country: Netherlands. Language: English.

AB A simple and rapid method of **purification** of restriction endonucleases from different Haemophilus strains is presented. By this method highly **purified** and stable enzymes can be obtained. Separation of different restriction activities present in the same strain is possible. This method was so far successfully used with Haemophilus influenzae, Haemophilus parainfluenzae and Haemophilus aegyptius strains. The main advantages over previously published procedures reside in the simplication of certain **purification** steps (for instance the BioGel A 0.5 M filtration is replaced by a **hydroxyapatite** batch step), elimination of exonuclease activity by fractionation with (NH4) 2SO4, separation of different restriction activities by phosphocellulose chromatography, application of this method to various strains and high **purification** degree of enzymes.

L24 ANSWER 91 OF 101 MEDLINE on STN
75097765. PubMed ID: 163367. Proteins in intracellular simian **virus** 40 nucleoportein complexes: comparison with simian **virus** 40 core proteins. Meinke W; Hall M R; Goldstein D A. Journal of virology, **(1975 Mar)** 15 (3) 439-48. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.
AB Intracellular nucleoprotein complexes containing SV40 supercoiled DNA were **purified** from cell lysates by chromatography on **hydroxyapatite** columns followed by velocity sedimentation through sucrose gradients. The major protein components from **purified** complexes were identified as histone-like proteins. When analyzed by electrophoresis in sodium dodecyl sulfate-polyacrylamide gels, complex proteins comigrated with **viral** core polypeptides VP4, VP5, VP6, and VP7. (3H) tryptophan was not detected in polypeptides from intracellular complexes or in the histone components from **purified** SV40 **virus**. However, a large amount of (3H) tryptophan was found in the **viral** polypeptide VP3 relative to that incorporated into the capsid polypeptides VP1 and VP2. Intracellular complexes contain 30 to 40% more protein than **viral** cores prepared by alkali dissociation of intact **virus**, but when complexes were exposed to the same alkaline conditions, protein also was removed from complexes and they subsequently co-sedimented with and had the same buoyant density as **viral** cores. The composition and physical similarities of nucleoprotein complex and **viral** cores indicate that complexes may have a role in the assembly of **virions**.

L24 ANSWER 92 OF 101 MEDLINE on STN
75097715. PubMed ID: 163342. Characterization of human papovavirus BK DNA. Howley P M; Mullarkey M F; Takemoto K K; Martin M A. Journal of virology, **(1975 Jan)** 15 (1) 173-81. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.
AB The DNA of the BK **virus** (BKV) human papovavirus was found to be heterogeneous, consisting of at least four discrete species of DNA. Only the largest of these four species, BKV DNA (i), which has a molecular weight calculated to be 96% that of simian **virus** 40 (SV40) DNA, was infectious. Homogeneous preparations of BKV DNA were obtained, however, from **virions purified** after low multiplicity infections of human embryonic kidney cells. BKV DNA (i) was shown to contain a single R-Eco RI and four R-Hind cleavage sites. The R-Eco RI site was localized in the largest R-Hind cleavage fragment. Radiolabeled BKV DNA reassociated slightly faster than SV40 DNA; 20 to 30% polynucleotide sequence homology was demonstrated between the genomes of BKV and SV40 when the reaction was monitored by chromatography on **hydroxyapatite**.

L24 ANSWER 93 OF 101 MEDLINE on STN
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(FILE 'HOME' ENTERED AT 18:36:36 ON 22 JUN 2005)

FILE 'USPATFULL' ENTERED AT 18:36:44 ON 22 JUN 2005
L1 9532 S HYDROXYAPATITE
L2 1599 S L1 AND HYDROXYAPATITE/CLM
L3 69 S L2 AND ADENOVIR?
L4 13 S L3 AND ADENOVIR?/CLM
L5 33 S L3 AND AY<2001
L6 27 S L5 NOT L4
L7 1 S US5496926/PN
L8 1 S L7 AND ADENOVIR?
L9 1 S US5624833/PN
L10 1 S L9 AND ADENOVIR?
E ERICKSON AMY E/IN
L11 1 S E3
E SHABRAM PAUL W/IN
L12 5 S E3

FILE 'WPIDS' ENTERED AT 18:46:16 ON 22 JUN 2005
L13 2918 S HYDROXYAPATITE
L14 14 S L13 AND ADENOVIR?
E SHABRAM P W/IN
L15 6 S E3
E ERICKSON A E/IN
L16 1 S E3

FILE 'USPATFULL' ENTERED AT 18:53:21 ON 22 JUN 2005
E VELLEKAMP G J/IN
L17 7 S E4 OR E5
L18 3 S L17 AND HYDROXYAPATITE
E CUTLER COLLETTE/IN
L19 1 S E3
E CANNON-CARLSON S V/IN
E CANNON CARLSON S V/IN
L20 5 S E4 OR E5

FILE 'MEDLINE' ENTERED AT 18:59:27 ON 22 JUN 2005
L21 9422 S HYDROXYAPATITE
L22 230 S L21 AND (VIR? OR RETROVIR? OR ADENOVIR?)
L23 199 S L22 AND PY<2001
L24 101 S L23 AND PURIF?

=> log off
ALL L# QUERIES AND ANSWER SETS ARE DELETED AT LOGOFF
LOGOFF? (Y)/N/HOLD:y
STN INTERNATIONAL LOGOFF AT 19:18:23 ON 22 JUN 2005